The chemical regulation of carcinogenesis.

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THE CHEMICAL REGULATION OF CARCINOGENESIS

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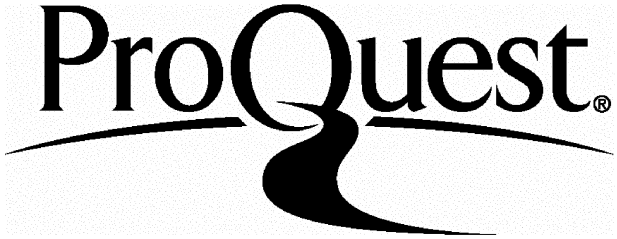

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for JENNY

SUMMARY

The work outlined in this thesis was conducted between October 1982 and September 1985, and is concerned with the use of chemical agents in the prevention and cure of cancer. Under this generally broad brief, we examined three topics of considerable interest in this field at the present time.

Firstly we investigated the role of three dietary indoles which have been shown to inhibit the induction of cancer by certain procarcinogens, in particular benzo[a]pyrene. In this work we prepared analogues of the dietary indoles with a view to ascertaining their mode of action and increasing their activity.

Next we devised a synthesis of dendrodoine, a natural product extracted in small quantities from the marine tunicate, Dendroda Grossular. This compound was shown in preliminary tests to be of potential use as a cytotoxic anti-cancer drug, but because of its inaccessibility its chemical synthesis was required if its properties were to be fully investigated.

Finally, we devised a new strategy in the synthesis of 6H-pyrido-[4,3-b]carbazoles. Although there has been a great volume of work carried out on these compounds over the last 25 years, there has yet to be devised a truly general synthetic route, which allows the functionalisation of these compounds at key points in their structure.

CONTENTS

Results and Discussion

CHAPTER 3: A New Route to 6H-pyrido[4,3-b]carbazoles

INTRODUCTION

INTRODUCTION

The term "cancer" is a generic name applied to a group of diseases which share a common type of disordered cell growth. The name itself is derived from the latin word for crab, and was originally used in this context to evoke the idea of a creeping ulcer, gradually spreading through the body, destroying everything in its path.

Few words in the modern day vocabulary incite such feelings of fear and suspicion among the general public as does the word 'cancer'. Popular imagination sees it as a disease which strikes indiscriminately and, defying all cures and treatment, advances slowly and unrelentlessly, causing extreme pain and discomfort, towards inevitable death. This irrational dread of the disease is largely a product of ignorance, resulting from the general lack of understanding of both its effects and causes, and fuelled by the social stigma associated with the disease.

These popular ideas surrounding cancer are frequently exaggerated, excessively pessimistic and often fail to take into account the recent advances in treatment and our understanding of the disease. On the other hand the fear of cancer is understandable given the extent of its proliferation in recent years. Throughout this century the death-rate from cancer has steadily risen to become today the second most frequent cause of death, after cardio-vascular disease, in the industrialised world. To put the problem in perspective, one in three people in Britain develop cancer at some stage in their lives, and one in five die from it. Although the dramatic increase in the incidence of cancer may largely be explained by the increased average life span of the population - a result of scientific advances in the eradication of infectious diseases - and the improved documentation of mortality, the general underlying trend is still upward.

There is strong medical evidence to suggest that a major percentage (up to 90%) of cancers are caused by environmental factors which include social and cultural habits, diet, agricultural practices and exposure to man-made pollutants. If these external agents can be identified and their mode of action understood, it should, in theory, be possible to prevent most, if not all, cases of cancer. Unfortunately financial and social pressure makes this approach to the problem far more difficult than it would at first seem, for in some cases, where these environmental elements have been identified, social and financial pressures have prevented their elimination from the environment. For example, lung cancer is largely caused by smoking; cancers of the large intestine and breast are associated with the consumption of "rich" foods; alcohol is related to cancer of the oesophagus; skin cancer is caused by exposure to ultraviolet light, and therefore by sunbathing; and cancer of the cervix has been associated with promiscuity. Until such time as governments and ultimately the public themselves can be convinced of the importance of preventing cancer through "healthy living", the main focus of research must be turned towards developing an understanding of the causes and effects of the disease and its ultimate treatment and cure.

The Origins and Effects of Cancer

All cancers originate as a defect in one or more of our cells. The human body contains roughly ten million million cells, sub-divided into groups and classes to form various tissues and organs. All cells contain the same genetic information and are programmed by the body to perform their various functions. The rate at which the cells of each group replicate varies. Nerve cells, for example, are apparently incapable of further division once they have been formed during the development of the

embryo; others such as liver cells only multiply after damage has occurred; yet another group of cells, including those of the skin and intestine, multiply constantly. In the latter case the rate of all multiplication must be closely controlled to match their rate of loss. This system of control is governed by some form of spatial restraint. For example, skin and intestinal cells exist in discreet layers and once a cell is forced outside this layer, it loses the ability to divide and undergoes a programmed series of changes ending in destruction.

Cancer cells differ from normally functioning cells in that they are not subject to the same spatial constraints and are thus able to expand without regulation. It must be pointed out that contrary to popular belief, cancer cells do not necessarily multiply at a faster rate than normal cells, but rather increase in population relative to that of normal cells because their growth is not regulated. Unrestrained growth of cancerous cells, if unchecked, will eventually develop into a cluster of cells forming a benign tumour at this, the primary, site. At this stage of development the animal is in no great danger provided the tumour is detected, as it may then be removed by surgery. However some, but not all, cancerous cells have the ability to invade the surrounding tissues and spread by way of the blood and lymph systems through the body to form secondary deposits (metastases). At this stage the tumour is said to be malignant. Normal cells fortunately do not undergo metastasis, being unable to reproduce once removed from the signal that governs their growth. Cancer cells can not only reproduce independently of these signals, but in some cases have the ability to dissolve proteins assisting implantation and attract a blood supply, thus ensuring unrestrained growth. In short, cancer cells are normal cells whose genetic programme has been somehow altered, allowing them

to function independently of the controls that govern the cells from which they originate.

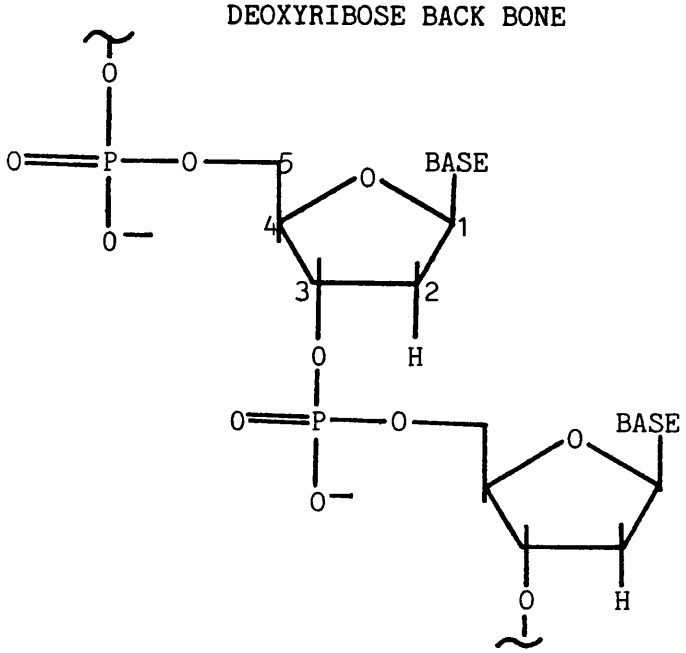
The behaviour of a cell is determined by the proteins it produces. Most of the chemical reactions in the cell are carried out by proteins called enzymes which bind together the partners of a chemical process on their surface and catalyse their reaction. Each protein consists of an unbranched single chain of one hundred or more amino acids joined in a fixed order. The activity of an enzyme depends on its three dimensional structure and the orientation of its "active" sites. These, in turn, are automatically governed by the exact sequence of amino acids in the enzyme chain. There are twenty amino acids used in protein synthesis, and the instructions that determine their order are contained in the deoxyribonucleic acid (DNA) of the cell nucleus.

DNA consists of two intertwined chains (the double helix). The back bone of each chain consists of the sugar unit deoxyribose, linked by a phosphate group from the 5-carbon atom of one deoxyribose residue to the 3-carbon of the next. Each sugar unit contains one of four bases: two purine, adenine (A)(1) and guanine (G)(2), and two pyrimidine, cytosine (C)(3) and thymine (T)(4), bonded to the 1-carbon atom (Figure 1).

The two strands of the double helix are held together by hydrogen bonding between the bases, ie the sugar phosphate back bone is on the outside of the helix and the bases on the inside. Also to maximise hydrogen bonding and minimise stereochemical interactions, adenine (A) only pairs with thymine (T), and guanine (G) with cytosine (C). Because of this specific base pairing, if the sequence of bases in one chain is known, eg -TCAT-, then the base sequence in the partner, or complementary chain can be deduced, namely -AGTA-.

FIGURE 1

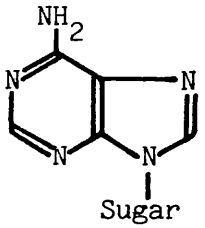
DEOXYRIBOSE BACK BONE



BASES

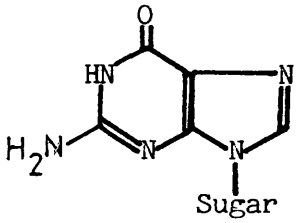
Purine

Adenine (A)



(1)

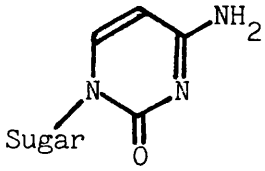
Guanine (G)



(2)

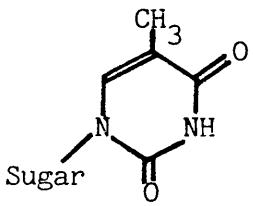
Pyrimidine

Cytosine (C)



(3)

Thymine (T)

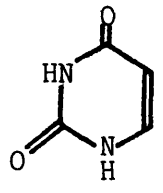


(4)

These bases can appear in any order in the DNA chain, their sequence being the coded information required for protein synthesis. A sequence of three bases (called a codon) dictates each amino acid in the sequence eg CAU is known to be the codon for the amino acid histidine. There are also stop codons used to end the amino acid sequence.

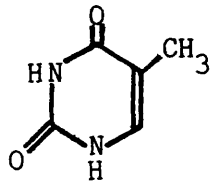
The stretch of DNA that contains the coded message for one protein is called a gene. Between the genes there are regions of DNA that do not code for a protein but are used for control purposes. These are called promoter regions and are used to turn the gene on and start the production of the protein concerned. The genetic code is translated from the DNA to the site of protein synthesis, the ribosomes, using a messenger molecule of ribonucleic acid (RNA). RNA is structurally very similar to DNA having a closely related phosphate back bone and four bases, three of which are present in DNA, namely guanine (G), cytosine (C), and adenine (A). The fourth base of RNA is uracil (U)(4a), which is chemically very similar to the missing DNA base thymine (T).

Uracil (U)



(4a)

Thymine (T)

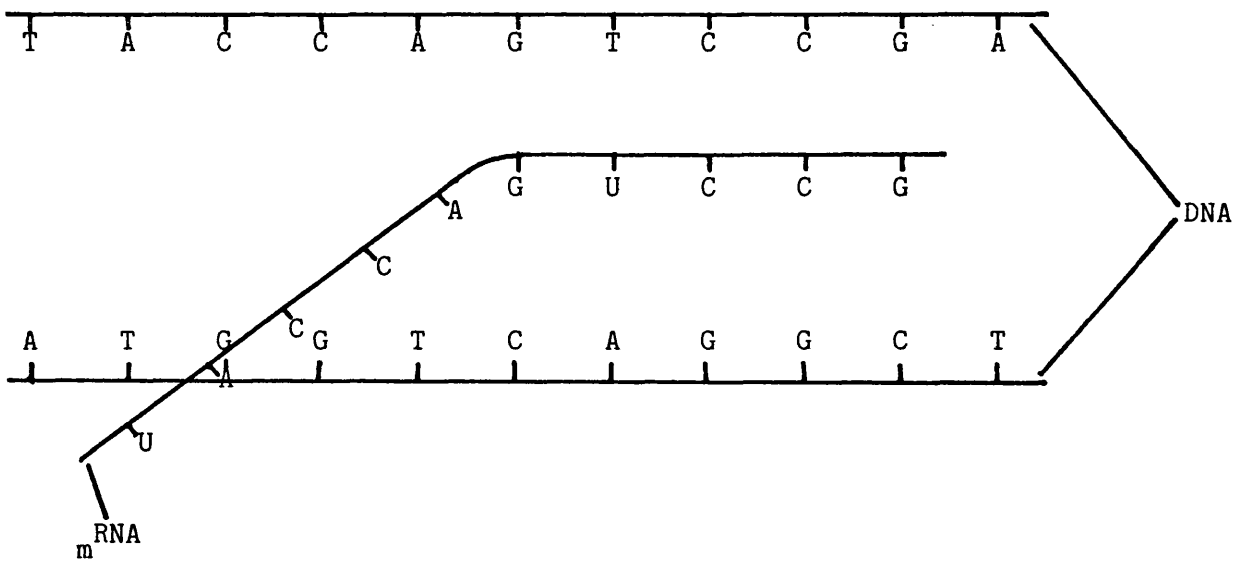


(4)

This messenger RNA ($_{m}$RNA) is synthesised by enzymes using one of the two DNA strands as a template forming the same base pairs as in the parent double helix, except that uracil (U)(4a) of RNA pairs with adenine (A)(1 of DNA. This process is called transcription (Figure 2).

FIGURE 2

The DNA Transcription Process



In turn the $_{m}$RNA molecule acts as a template for amino acid sequencing to form proteins. This process is known as a translation.

DNA ——TRANSCRIPTION——>> RNA ——TRANSL ATION——>> PROTEIN

$_{m}$RNA is unstable and therefore its constant production is required if a protein is to be continuously synthesised. The mode of action of a cell can therefore be altered if the type and quantity of the messengers produced changes, ie which genes are expressed and which are repressed. This gene control is carried out by special proteins which interact with the promoter regions of the DNA previously mentioned. It is a fault or mistake in this control mechanism causing the expression or repression of key genes, which gives cancerous cells their abnormal properties.

When a cell divides, the DNA chains of the double helix separate and build new identical complementary chains using the enzyme DNA polymerase. If any changes occur in the base sequence of a chain, called a mutation, then on replication the error will be copied and therefore handed down to subsequent generations.

The simplest example of a mutation is where one base is changed for another, thus causing a change in the coded message (the codon) of which it is a member. If the codon is converted in a stop codon, the protein will be truncated and thus deactivated. Changing the codon to that of another amino acid, on the other hand, can make no difference to the activity of the protein concerned, unless it is at an important point of the protein, eg the active site of an enzyme.

Fortunately, the cell carries two copies of most genes (one paternal and one maternal), so if one gene copy is affected, the other can produce enough of the normal protein for the needs of the cell. Mutations of this type are called recessive, as they require both copies of the genes to be affected for problems to occur. If, however, the mutation occurs in the promoter region between the genes permanently activating a normally repressed gene, the cell will continuously produce a protein unnecessary for its normal function. This is said to be a dominant mutation requiring only one of the two gene copies to be affected.

A second, more extensive, type of mutation may also occur if one or more bases are added or removed from the DNA sequence, causing a potential "frame-shift" to occur. A simple example of the dramatic effect, which the addition of just one base can have, is shown below:

Correct message: SHE SAW THE CAT

Frame shift (* base added): SH* ESA WTH ECA T

Not surprisingly, a "frame-shift" almost invariably destroys the function of the gene in which it occurs.

A third type of mutation, known as a large scale rearrangement, can also occur where long sequences of DNA are deleted, inverted or moved from one region to another. If, for example, a normally repressed gene is translocated to a site near an active promoter region causing it to be activated, a dominant mutation can occur.

Environmental Factors

Although mutation can occur spontaneously, it is far more often caused by interaction of the DNA with environmental agents, called mutagens, such as radiation, chemicals and viruses.

1) Radiation:

Both electromagnetic radiation (eg ultraviolet light and X-radiation) and particulate radiation (eg electrons and neutrons) can damage DNA, cause mutations and lead to cancer. X-radiation tends to produce breaks in the DNA chains, causing large scale rearrangements. Ultra-violet light, on the other hand, has been shown to fuse adjacent thymine residues together. Normally these lesions are quickly excised and replaced, but if the cell replicates before this repair can take place, the new

strand of DNA may possess incorrect bases opposite one or both bases of thymine "dimer", as the DNA polymerase may not recognise them in their fused form (figure 3a).

2) Chemicals:

Chemical agents have a wide range of effects on DNA. They can, for example, cause the addition or removal of substituents on the bases, produce cross-linking between the two chains of DNA or link themselves into the DNA chain. The action of two common chemical mutagens are discussed below.

(a) Ethyl methanesulphonate (EMS)

EMS acts by O-methylating the guanine base of DNA at position-6, which then tends to be mistaken for adenine when the DNA is subsequently replicated. EMS therefore converts a GC base pair into an AT pair (figure 3b).

(b) Benzo[a]pyrene B[a]P

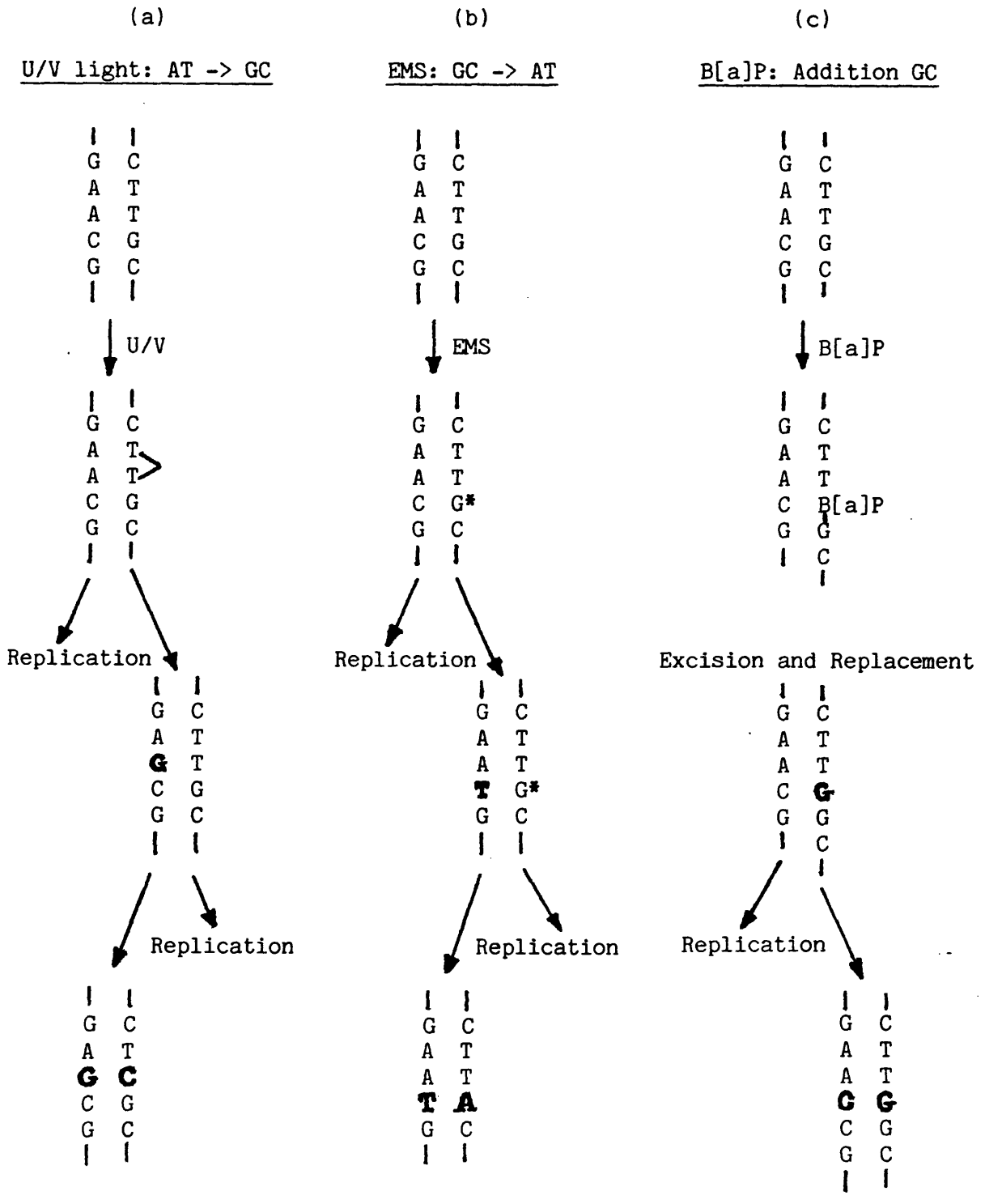
B[a]P, after metabolism to its mutagenic form (see chapter 1) can align itself into the DNA molecule as if it were a base, and bond to an adjacent guanine residue. During the excision and replacement of this guanine-B[a]P complex, the insertion of an extra guanine base may cause a frame-shift (figure 3c).

3) Viruses:

A number of viruses have been identified which induce tumours under both natural and experimental conditions. Further investigations of some representative tumour viruses has shown them to implant one or more genes onto the host DNA. Although the function of the resultant viral coded product from these "new" genes is largely unknown, it may inadvertently change the intrinsic properties of the host cell causing it to become cancerous.

FIGURE 3

Effects of Various Mutagens on DNA



Treatment

In essence there are currently three therapeutic weapons available to combat cancer, namely surgery, radiotherapy and chemotherapy, although great interest has recently been expressed in the techniques of immunotherapy. The merits of each method of treatment are discussed briefly below.

1) Surgery:

Surgery is still the major course of treatment for cancer. It is most effective if carried out before the tumour has become malignant. However, as not all metastases develop into new tumours, the removal of the tumour shortly after metastasis has occured may also effect a cure by eliminating the source of invasive cells and therefore preventing the increase in secondary sites. One major argument against surgery is the belief that the operation itself may be responsible for causing metastasis by dislodging small groups of cancerous cells into the circulatory systems of the body.

2) Radiotherapy:

Radiotherapy is the second most widely used form of treatment for cancer and is normally used in conjunction with surgery, or when a tumour, because of its position, cannot be surgically excised. In radiotherapy, the tumour and its surroundings are exposed to ionising radiation which interacts with water, and subsequently oxygen, in the cell to form radicals. It is these radicals which cause cellular damage, resulting in cell death. Similar damage is inflicted on both tumour and normal cells, but the latter, due to their slower growth rate, have a greater ability to repair the damage and thus recover. As a result,

when the affected area is repeatedly exposed to radiation, at intervals of normally one or two days (known as "fractional dosage"), the tumour undergoes greater net cell loss.

Another reason for cancer cells' vulnerability to radiation is that they are generally better oxygenated than normal cells and, as oxygen assists in the radical damage process, cancer cells will incur more damage than surrounding cells from the same incident of radiation. This process is called radiosensitization. Attempts to provide oxygen substitutes that can be selectively diffused into the tumour and sensitize them to radiation by either increasing the damage caused or affecting the repair process have recently acquired impetus. In addition, radioprotective agents are also being developed, designed to reduce radical damage in normal cells.

Despite these advances, however, normal cell damage and unpleasant side effects, such as nausea, vomitting and loss of hair, are still a major problem with the use of radiotherapy.

3) Chemotherapy:

Although there is increasing interest in the use of drugs as inhibitors of carcinogenesis (see chapter 1), at present the drugs used in chemotherapy are primarily cytotoxic in nature, and work by inhibiting the normal functions of the cell, particularly the process of cell division. The degree of selectivity of these agents for cancerous cells (cf normal cells) varies according to their mode of action. Three of the most commonly used types of chemotherapeutic agents, namely alkylating agents, antimetabolites and DNA intercalators are discussed below.

a) Alkylating Agents:

These agents, of which nitrogen mustard (5) is the simplest example, act by alkylating the nucleic acids of the DNA causing genetic

miscoding, loss of activity of major genes and subsequent cell death. As with radiation therapy, these drugs kill both cancerous and normal cells alike, but since the latter are not continuously dividing, they have more time to effect repairs, thus having a higher survival rate.

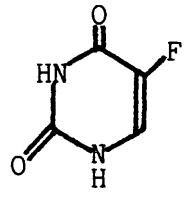
$$CH_3N(CH_2CH_2Cl)_2$$

(5)

b) Antimetabolites:

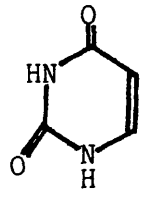
This group of agents are analogues of naturally occuring metabolites, which inhibit the enzymatic pathways of the cell and are incorporated into macro-molecules. One of the simplest examples of this type of agent is 5-fluorouracil(4b), which is an analogue of one of the RNA bases, uracil (4a). When present in a cell, 5-fluorouracil (4b) may be mistakenly incorporated into RNA causing errors in the resultant protein synthesis. The high selectivity of these agents for proliferating cells (cf to resting cells) arises from their greater metabolite requirements, and therefore antimetabolite uptake and usage.

5-Fluorouracil



(4b)

Uracil



(4a)

c) Intercalators:

Intercalators cover a wide range of compounds, including ellipticine, olivacine and the antibiotics dactinogycin, adriamycin, daunorubinic and mithramycin, as used in cancer treatment. They operate by interacting with the stacked DNA bases creating a distortion or local unwinding of the DNA helix, and thus interfere with the function of DNA and RNA polymerases, which require correct orientation and separation of the two strands for their activity. The site of interaction can either be on the DNA bases or sugar phosphate back bone, and differs from drug to drug.

Steroid hormones have also been used in chemotherapy on an empirical level. In breast cancer, for example, estrogen may either stimulate or inhibit tumour growth.

Although at present chemotherapeutic agents suffer the same drawbacks as radiotherapy, much hope for the future rests with this form of treatment. As the subtle differences between the vital processes of cancerous and normal cells are recognised and understood, so the criteria for drug design should become more specific and the selectivity of the drugs increase.

4) Immunotherapy:

Immunotherapy, as the name suggests, makes use of the body's own immune system to combat the proliferation of cancerous cells.

All cells have sites on their surface called antigens, which the body's immune system uses as markers. Cells alien to the body have alien antigens and it is the presence of these alien antigens which enables the body's immune system to recognise the alien cells and destroy them. As cancer cells have different properties to those cells from which they originate, they may also possess "alien" antigens.

If these alien antigens can be recognised and isolated, then the body's defences can be specifically raised against them. This method is at present still at the research stage, and there are still many problems to overcome, but if perfected, it would allow the innoculation against specific cancers, as is presently possible in the case of many infectious diseases.

Prospects for the Future

Although great advances have been made over the last 20 years in the field of cancer research to the point where today it can be said that there is finally a glimmer of light at the end of what previously seemed to be an endless tunnel, considerable work is still necessary before cancer can be regarded as vanquished, if this is indeed possible.

As the maxim says, "prevention is better than cure", and it is in this area that a greater and more concerted effort is needed, not only on the part of the medical profession, but also by means of governmental pressure to educate the public to the way in which they themselves can actively take measures to reduce their risk of developing cancer. Indeed, some progress has already been made in this area; the increasing trend towards a "healthy", high fibre diet and the steadily growing view of smoking as a "dirty", anti-social habit are two examples of just how successful such attempts at re-educating the public can be.

However, the day when all carcinogens have been recognised and eradicated from our environment, if that can ever be the case, is still a long way off, and in the meantime millions of people are suffering and dying of cancer. Therefore research is still needed in the field of treatment and cure. As the efficiency and availability of screening techniques improve, so many more cancers will be detected at an earlier

stage of development, hopefully when the cancer is still benign and when surgical excision often affords a complete cure free from any side effects.

Although improved screening techniques may relieve the problem, they cannot be considered as offering a complete cure, as many cancers develop metastases at a very early stage in their development, before detection is possible, and therefore improvements in chemotherapeutic techniques and the development of immunotherapy still have a vital role to play.

As the specificity of chemotherapeutic agents increases, so the harmful side effects caused by normal cell death, which have to date been a major drawback with this form of treatment, could be at least minimised, if not completely eliminated. If immunotherapy lives up to its potential, it may also provide a complete and harmless cure for the disease.

At the present time, there would seem to be a certain air of optimism in the field of cancer research, and provided that funds remain available for the vital research to be carried out, there would seem to be no reason why the techniques in the treatment of cancer should not steadily improve until cancer as a major cause of death has become a thing of the past.

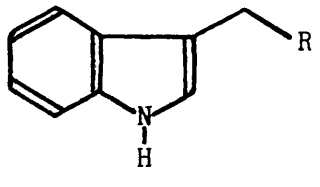
C H A P T E R 1

Dietary Indoles

INTRODUCTION

It has been known for some years that the ingestion of certain compounds present in our diet is responsible for many of the cancers of the dietary tract. Recently, however, an increasing number of other structures have been shown to inhibit the action of these carcinogens and therefore prevent the onset of cancer. These inhibitors are presently of great interest as, unlike the drugs presently available which attempt to cure the disease, they could be used as prophylactics and prevent cancer in the first place. Such compounds are also not necessarily cytotoxic in their mode of action and so do not possess the same unpleasant and harmful side effects still associated with the best of the presently used chemotherapeutic agents.

Among these inhibitor compounds three indoles, namely indole-3-acetonitrile (6), 3,3'-diindolylmethane (7) and indole-3-carbinol (8), are of particular interest, being not only simple in structure but also common dietary substituents.

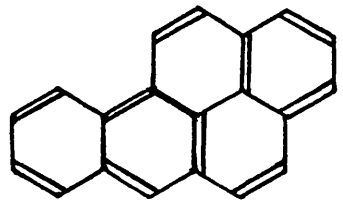


(6) R=CN

(7) R=3-indolyl

(8) R=OH

These three simple indoles are found in cruciferous vegetables (Brassicas), such as sprouts and cabbages, and have been shown in both in vitro and in vivo experiments to inhibit the development of cancers induced by certain polycyclic aromatic hydrocarbons (PAH), including benzo[a]pyrene (B[a]P)[1] (9), and some other environmental carcinogens, such as N-nitrosodimethylamine[2] (NDMA) (10).



B[a]P (9)

$(CH_3)_2N\text{-}NO$

NDMA (10)

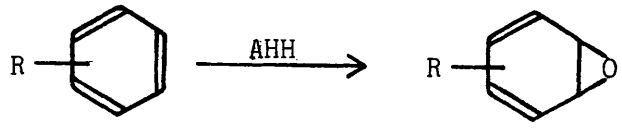
The role of polycyclic aromatic hydrocarbons (PAH) as environmental agents of carcinogenesis

Polycyclic aromatic hydrocarbons (PAH) are produced mainly by the combustion of organic matter and are present in varying quantities in most elements of our environment eg air, soil and water, as well as being a major constituent of cigarette smoke. Medical evidence has shown some

of these polyaromatic compounds to be highly carcinogenic and their effects are linked to many cancers, including those of the lung, intestine, breast and skin. As the reliance of the industrialised world on fossil fuels as a source of power continues, so the production of this group of environmental carcinogens is also guaranteed.

Polycyclic aromatic hydrocarbons and indeed many other environmental carcinogens such as N-nitrosodimethylamine (NDMA) (10) are not strictly carcinogens, but rather procarcinogens, as they require metabolic activation before they become a threat to the host. Ironically this biological activation is often effected by one of the body detoxification processes, which are designed to remove unwanted and potentially toxic compounds from the body.

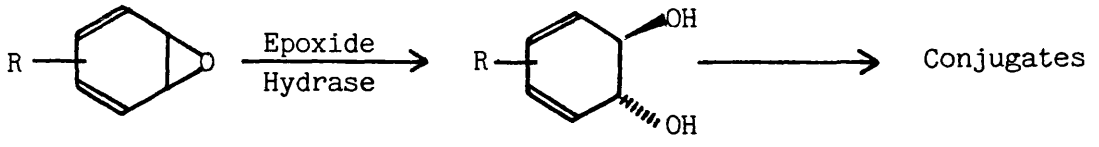
Aromatic hydrocarbons and many other potentially toxic compounds present in the blood supply are hydroxylated in the liver by the microsomal monooxygenase system to render the compounds more water soluble and so facilitate their excretion from the body. This microsomal monooxygenase system is a multi-enzymes electron transfer system containing cytochrome p450 which acts by complexing molecular oxygen and transferring it into the substrate molecules. In the case of PAHs, the enzyme called aromatic hydrocarbon hydroxylase (AHH) initially converts the aromatic substrate into an arene-oxide (Scheme 1).



Arene Arene-oxide

SCHEME 1

The arene-oxide intermediate is relatively reactive and may suffer various fates. In one of the further detoxification processes it is converted to the corresponding trans-diol, using the enzyme epoxide hydrase, and is subsequently conjugated to a hydrophilic agent, such as glucoronic acid and excreted from the body (Scheme 2).



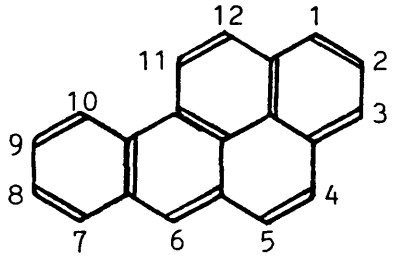
SCHEME 2

There are several other conjugating enzyme systems which also facilitate excretion of the arene-oxide in a similar manner. Alternatively it may be converted to the corresponding phenol by an acid catalysed non-enzymatic process.

Fortunately none of the above fates endanger the host organism in any way. If, however, the arene-oxide is not rapidly metabolised by the processes mentioned above it may react with intra-cellular nucleophiles, including DNA or RNA and essential proteins, potentially initiating carcinogenesis.

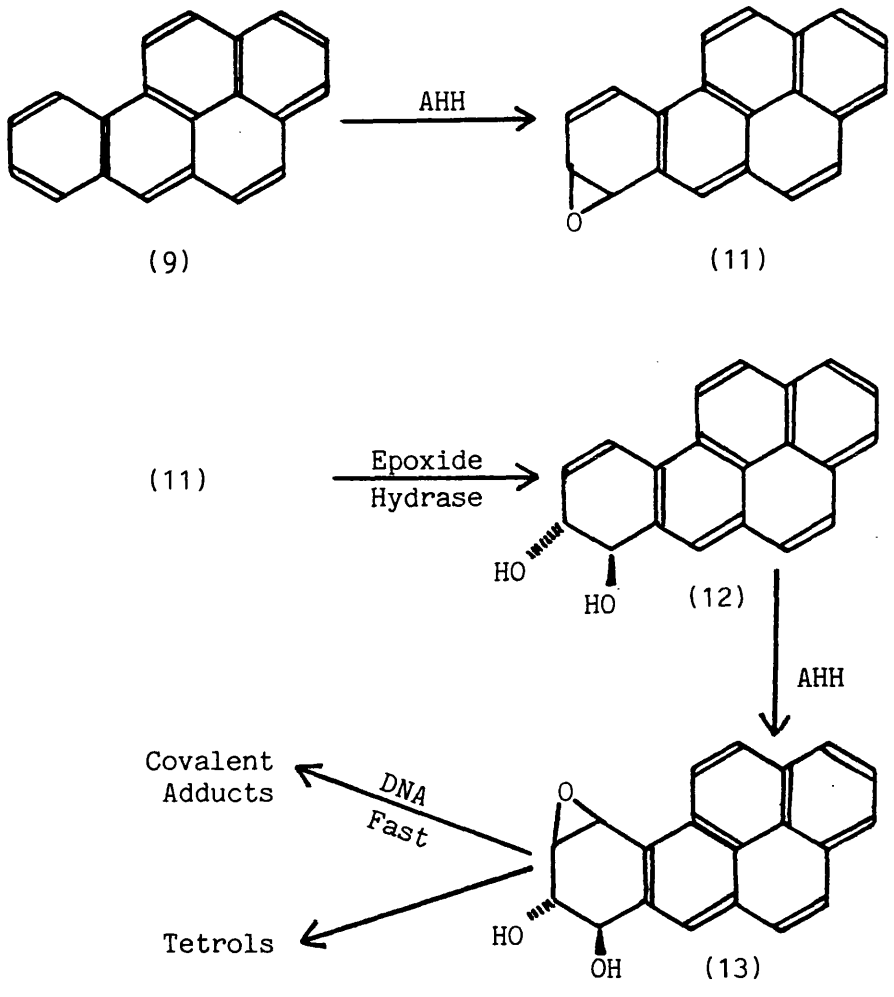
Although all PAHs are believed to undergo similar detoxification processes, not all are procarcinogens; in fact, the property is limited to a minority of the many hundreds of arenes known. Whether or not an

aromatic compound is a procarcinogen depends on the relative rates of the further detoxification processes and DNA-binding of the arene-oxide. Of the known procarcinogens, benzo[a]pyrene (9) is one of the most prevalent being the principal carcinogenic agent in coal tar.



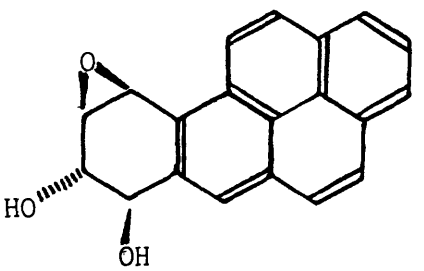
Benzo[a]pyrene (9)

Benzo[a]pyrene (9) is converted by the liver microsomes to several epoxides and phenols of which the 7,8-epoxide (11) is a major product. This intermediate (11) is then ring opened by the enzyme epoxide hydrase to the corresponding trans-7,8-diol (12). If this diol is not removed from the body by a further detoxification process, it may undergo a second epoxidation to yield the 7,8-dihydroxy-9,10-epoxide (13). This diol epoxide (13) is highly reactive toward nucleic acids and proteins forming covalent adducts. It is significant that reaction with nucleophiles is faster than the competing enzymatic conversion to the corresponding tetrol, which is not toxic and can be excreted in the form of conjugates (Scheme 3).



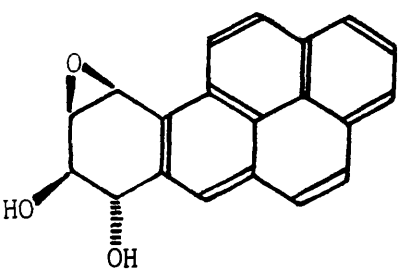
SCHEME 3

It is this 7,8-dihydroxy-9,10-epoxide of B[a]P (13) which is the ultimate carcinogen. There are two distinct geometrical isomers of the species (13): the "syn" isomer (13a), where the 7-hydroxy group is cis to the epoxide oxygen atom, and the "anti" (trans) isomer (13b), where the relationship is reversed.



SYN

(13a)

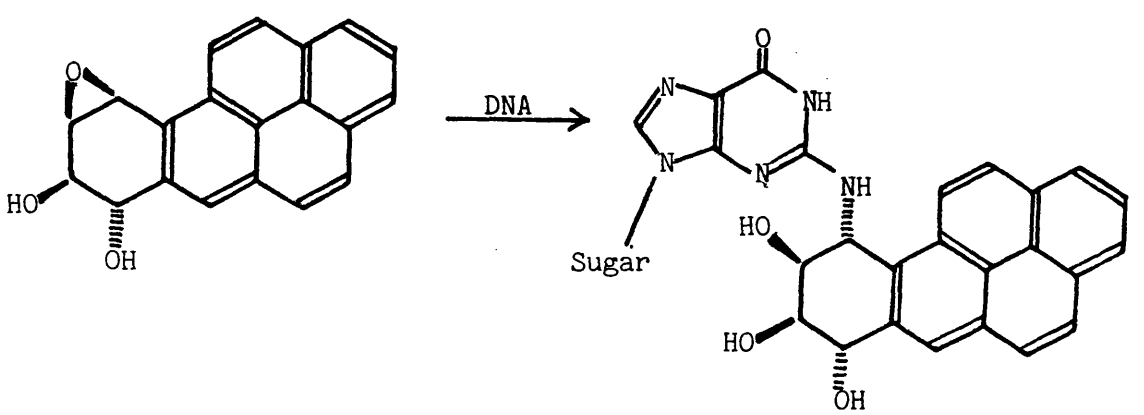


ANTI

(13b)

Work by King et al[3] has shown that the "anti" isomer is mainly responsible for DNA binding, although a small contribution from the cis form also occurs.

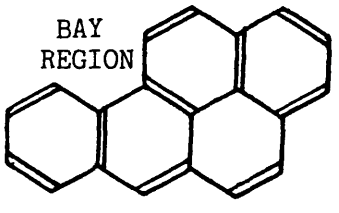
The major site of interaction of the anti B[a]P diol epoxide (13b) with DNA was established by Oxborne[4] as being the 2-amino group of the guanine base (Scheme 4), although products involving unions with other positions on guanine, adenine and cytosine were also suspected as playing minor roles.



SCHEME 4

Attempted natural repair of this guanine-B[a]P adduct is often inaccurate and causes genetic misinformation and hence mutation to occur (see page 10).

One recently proposed reason for the increased activity of the diol-epoxide (13) toward DNA (cf to other arene-oxides) and hence its highly mutagenic properties is that it contains a "bay-region" epoxide. The bay-region refers to that portion of the molecule where an angular fusion of a ring to a linearly arranged polycycle creates a "bay" of space surrounded by a π-system.



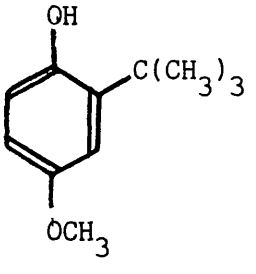
Molecular orbital calculations on these systems imply that an epoxide formed in this region would readily open to a stabilised carbonium ion and hence enter readily into S_N1 alkylation processes with nucleic acids.

The action of indole-3-methylene inhibitors

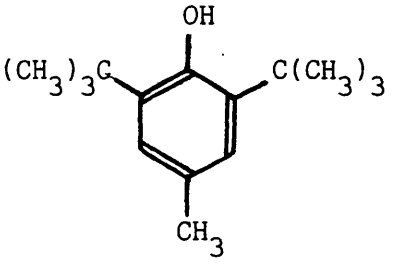
As previously mentioned, indole-3-acetonitrile (6), 3,3-diindolylmethane (7) and indole-3-carbinol (8) are present in cruciferous vegetables and have been shown to inhibit the induction of cancer by benzo[a]pyrene

B[a]P (9). The three indoles have been tested[1] for their effects on B[a]P (9) induced neoplasia in rodents: when added to the diet all three were found to inhibit B[a]P-induced neoplasia of the forestomach and pulmonary adenoma formation. In other experiments indole-3-carbinol (8) and 3,3'-diindolylmethane (7) were also found[5] to inhibit 7,12-dimethylbenz[a]anthracene DMBA (another PAH procarcinogen) induced tumours, but indole-3-acetonitrile (6) was inactive.

The original rationale for the activity of these three simple indoles was their known ability to alter the activity of the microsomal monooxygenase system. This property has been studied extensively for two phenols, butylated hydroxyanisole (BHA) (14) and butylated hydroxytoluene (BHT) (15) which are used as antioxidant food additives.

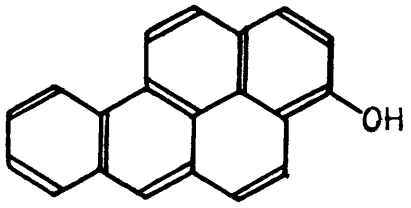


(14)



(15)

With these phenols it is known that alterations of this enzyme system can change the metabolite pattern of PAH procarcinogens. It has been shown, for example, that BHA (14) reduces the epoxidation of B[a]P and increases the formation of 3-hydroxybenzo[a]pyrene (16), a non-carcinogenic metabolite.



(16)

Both phenols have also been shown[7,8] to increase the activity of the conjugating enzyme systems and hence increase the overall rate of complete detoxification.

It is probable that the induction of this enzyme system brought about by these dietary indoles is due to their own oxidation and their role may be as "suicide" substrates. For example, these dietary indoles may be oxidised in preference to the procarcinogens by the microsomal enzymes so that the ultimate carcinogens are not produced, in the same way that ethanol is preferrentially oxidised in the presence of methanol. Alternatively, they or their metabolites may act as radical scavangers: radicals have often been shown to mediate carcinogen production, and Shertzer et al[9] have shown, in tests on indole-3-carbinol (8), that there is a high correlation between the reduction in lipid peroxidation, caused by free radicals, and DNA binding of the procarcinogen, NDMA (10).

A third possibility recently suggested by Shertzer[2] is that the metabolites of these indoles interact directly with the carcinogens in a detoxification process.

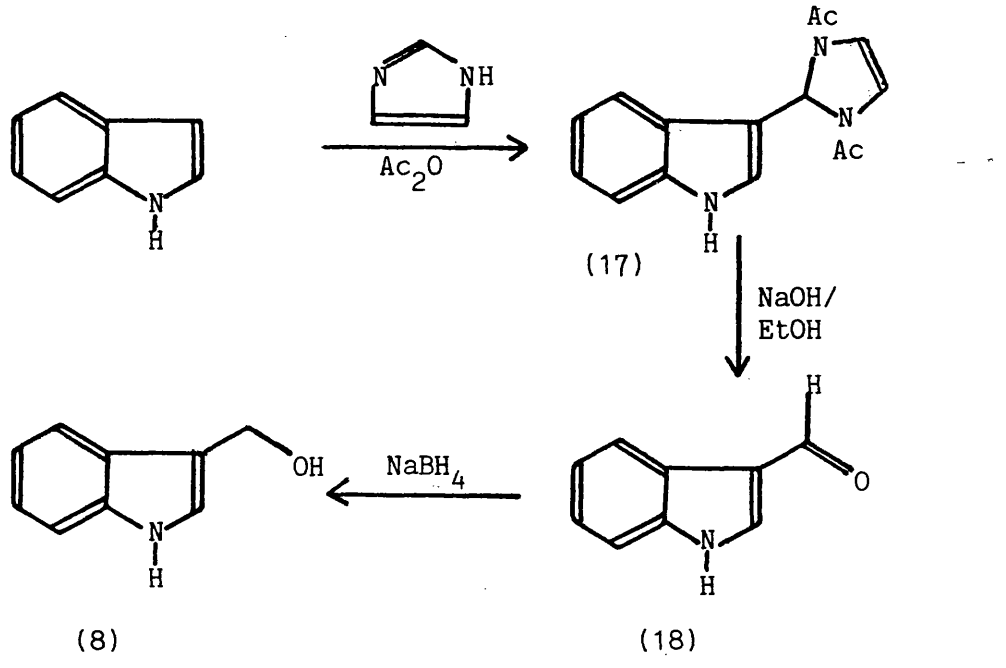
It is quite apparent that a true understanding of the mode of action of these dietary inhibitors is required, not only as a guide in optimising

their activity, but also to highlight any potential hazards, which the long term administration of these drugs could cause. For example, if these compounds do effect the metabolic pattern of B[a]P (9), they may also affect the metabolic pattern of other normally non-carcinogenic compounds producing carcinogens or toxins. A competitive oxidation process, on the other hand, may prevent the excretion of potentially harmful compounds from the body.

RESULTS AND DISCUSSION

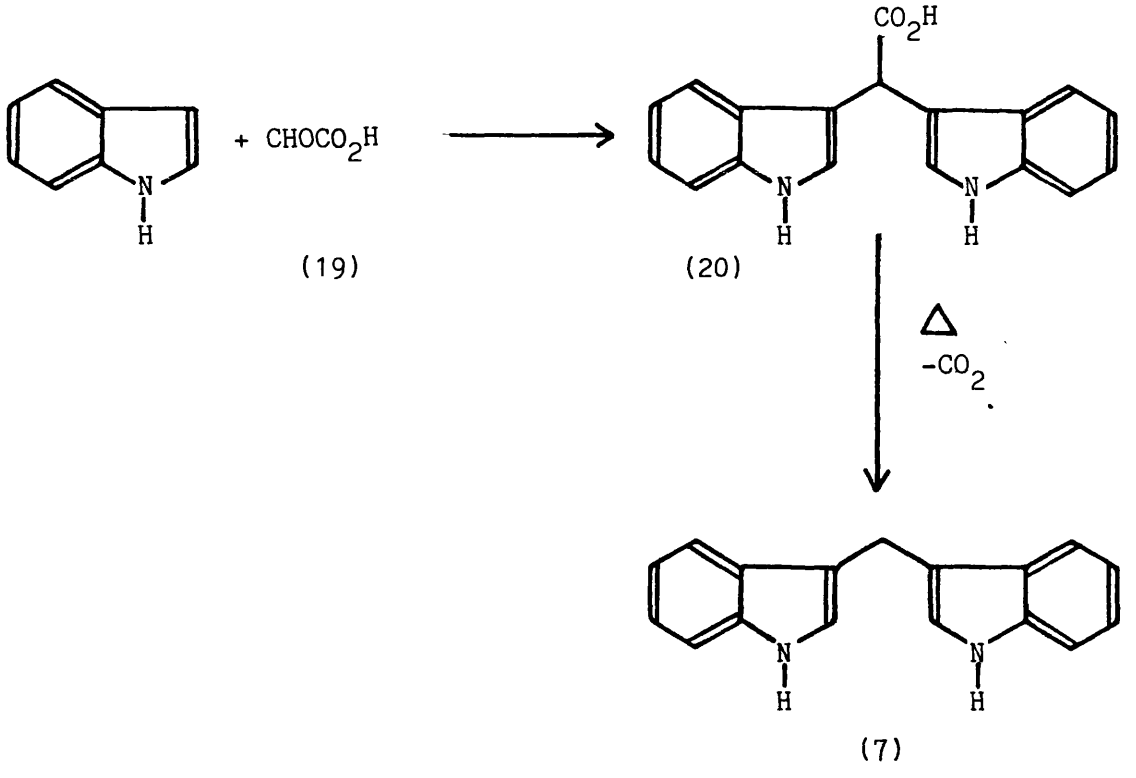
Although the potential value of these compounds cannot be understated, it was clear from an extensive search of the literature and correspondence with one of the leading researchers in the field, namely Dr L W Wattenberg, that no synthetic work had so far been carried out to modify the dietary indoles (6), (7) and (8). We therefore set about the synthesis of analogues of these simple indoles with a view to optimising their activity and thus hopefully to obtaining clues to their mode of action.

The first step was to obtain samples of the three dietary indoles for comparative testing. Of the three, only indole-3-acetonitrile (6) is commercially available, but the other two were readily synthesised by reported literature methods: indole-3-carbinol (8) was prepared by the reduction of 3-formyl-indole (18) using sodium borohydride[10]. The starting material was in turn synthesised from indole[11] via the imidazoline derivative (17) (Scheme 5).



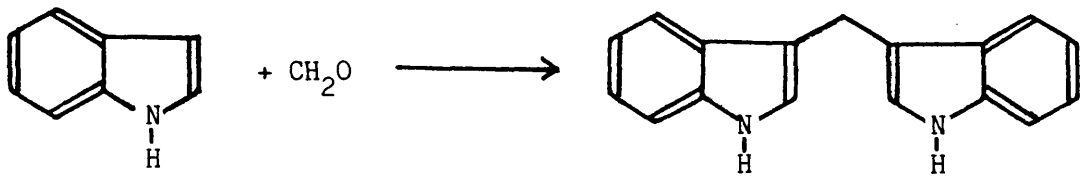
SCHEME 5

The first literature method for the synthesis of 3,3'-diindolylmethane (7) which we attempted was that reported by Julia and Tilly[12]. Here indole was reacted with glyoxylic acid (19), yielding 3,3'-diindolylacetic acid (20). On heating to 195°C, this acid (20) was said to decarboxylate to the required diindolylmethane in 60% yield (Scheme 6).



SCHEME 6

In our hands, however, the decarboxylation step repeatedly yielded an intractable tar which could not be resolved into pure product, and so the method was abandoned. Next we attempted a similar synthetic route proposed by Thesing[13], where 3,3'-diindolylmethane (7) was synthesised directly from the condensation of indole and aqueous formaldehyde (Scheme 7).

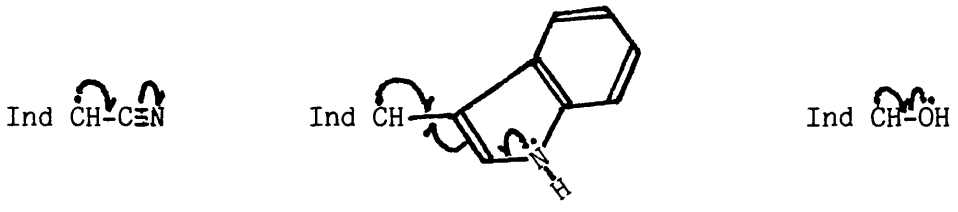


(7)

SCHEME 7

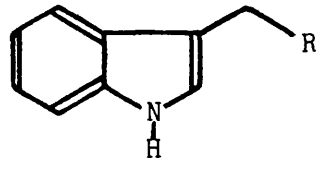
We were pleased to find that this reaction successfully afforded the required 3,3'-diindolylmethane (7) in 81% yield.

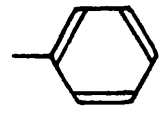
We next turned our attention to the design and synthesis of analogues of these simple indoles. Although the exact biological mode of action of these indoles is unknown, it would seem likely that their radical oxidation is at least part of the inhibitory process. With this in mind we examined the three dietary indoles for potential oxidation sites, and observed that they all possessed substituents which could stabilise a radical initially sited on the methine position.

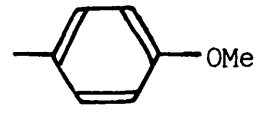


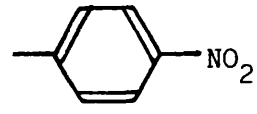
(6) (7) (8)

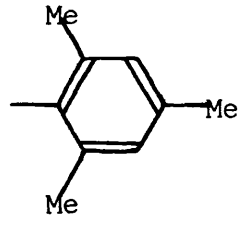
Our original strategy was therefore to synthesise indole-3-methylenes with the ability to stabilise a methine radical. The classic method of stabilising radicals is by the introduction of an adjacent electron donating, an electron withdrawing, a conjugated system or a bulky substituent. To test each of these criteria and therefore the viability of our original assumption, the following compounds were targeted:-



(21) R = 

(22) R = 

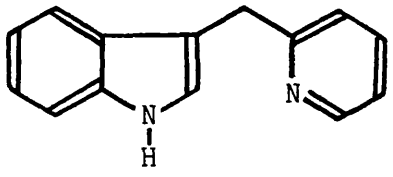
(23) R = 

(24) R = 

(25) R = H

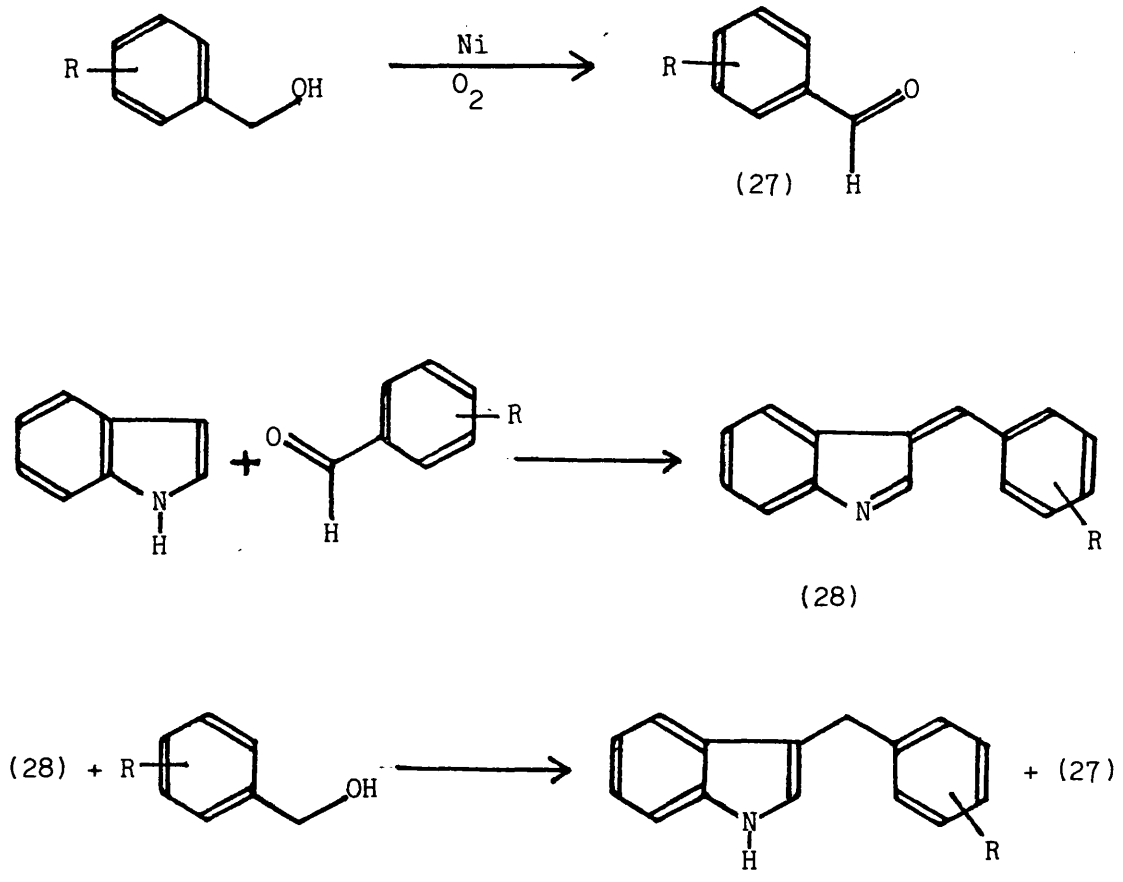
3-(4-methoxybenzyl)indole (22), where R is electron donating;
3-(4-nitrobenzyl)indole (23), where R is electron withdrawing;
3-(2,4,6-trimethylbenzyl)indole (24), where R is bulky;
3-benzylindole (21), where R is simply conjugated; and finally skatole (25) to check the necessity of the aryl substituent.

On searching through the literature, no reference could be found to any 3-(nitrobenzyl)indole derivatives and preliminary attempts to synthesise them by the usual methods failed, so 3-(2-pyridinylmethyl)indole (26) was chosen as an alternative example of an indole-3-methylene derivative with an electron deficient substituent.



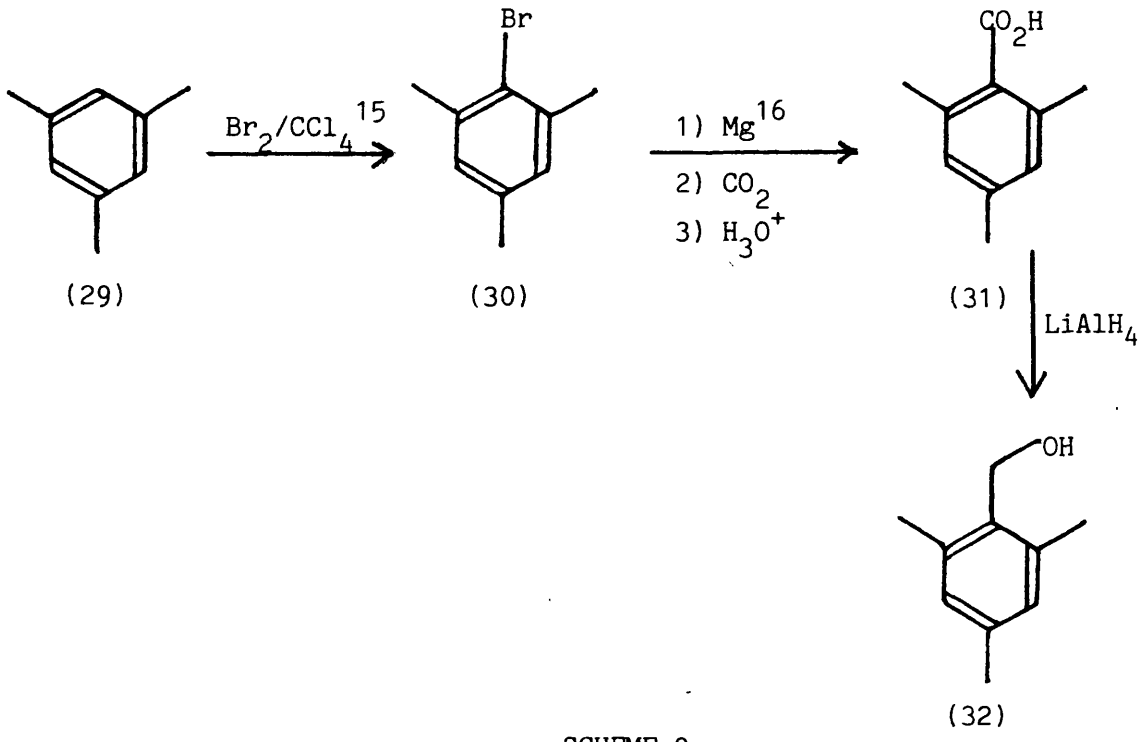
(26)

Skatole (24) was commercially available. The four other compounds were synthesised by a method developed by Pratt and Botimer[14] for the general synthesis of alkyl and aryl substituted indole-3-methylene compounds, including 3-benzylindole (21) and 3-(4-methoxybenzyl)indole (22). In this method indole is refluxed in p-cymene with a trace of nickel, potassium hydroxide and an excess of the relevant benzyl alcohol. The nickel metal is assumed to catalyse the oxidation of a small amount of the alcohol to the corresponding aldehyde (27), which is subsequently attacked by either indole or its potassium salt. The resultant adduct then dehydrates to the indolenine intermediate (28), the subsequent reduction of which to the required indole promotes oxidation of more of the alcohol to the aldehyde (Scheme 8).



SCHEME 8

Only 2,4,6-trimethylbenzyl alcohol (32) of the four alcohols required for use in this method was not readily available (and is relatively expensive to buy), so it was synthesised from mesitylene (29) by the following route (Scheme 9).



SCHEME 9

In our hands the implementation of the above "indole" synthesis yielded the required structures in the following yields:-

3-benzylindole (21) - 75%

3-(4-methoxybenzyl)indole (22) - 33%

3-(2-pyridinylmethyl)indole (26) - 33%

3-(2,4,6-trimethylbenzyl)indole (24) - 10%.

This is the first recorded synthesis of 3-(2,4,6-trimethylbenzyl)indole (23). Although the yields of these reactions were only moderate, except for 3-benzylindole (21), the reactions were carried out on a reasonably large scale, so that sufficient of each compound was obtained for biological testing.

After purification, these four target compounds, (21), (22), (24), (26), together with skatole (25), indole, the three dietary indoles (6),(7) and (8), and a sample of 3-(3-pyridinylmethyl)indole (33), a compound previously synthesised in these laboratories, were all sent to Professor H Shertzer at the University of Cincinnati for biological appraisal.

The compounds were tested in vitro for their ability to prevent phospholipid peroxidation, a property which Shertzer has linked to cancer inhibition, using two separate oxidation systems:-

a) Iron/ascorbic acid (Fe/Asc), an aqueous system,

b) Azobisisobutyronitrile in chlorobenzene (AIBN/CB), a hydrophobic system where oxidation is initiated by the thermal decomposition of AIBN.

The system of testing was adopted because phospholipid peroxidation is easily monitored and has been shown by Shertzer[9], using indole-3-carbinol (8) as the substrate and NDMA as the procarcinogen, to be closely related to the rate of DNA binding of the ultimate carcinogen.

The compounds were each tested at increasing concentrations for their ability to reduce lipid peroxidation relative to a control where no inhibition was used (ie 100% control = no inhibition, 0% control = 100% inhibition) and the results were plotted for both oxidation systems. The compounds were also ranked by the concentration of them required to bring about 50% inhibition of lipid peroxidation. The biological testing procedure is fully discussed in Appendix 2. The results obtained for the compounds originally tested are given below in Table 1.

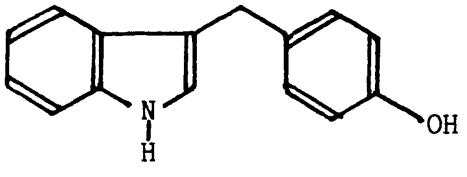
TABLE 1

Biological data on dietary indoles and original target compounds

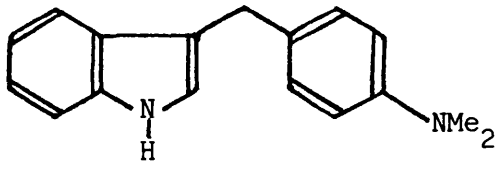
Compounds	Oxidation system Conc. 50% I μm (Ranking)	
	AIBN/CB	Fe/Asc
3-(2,4,6-trimethylbenzyl)indole 424)	100(2)	11(1)
3,3'-diindolylmethane (7)	64(1)	15(2)
3-(4-methoxybenzyl)indole (22)	300(6)	24(3)
3-benzylindole (21)	185(4)	36(4)
indole-3-carbinol (8)	120(3)	300(5)
3-(2-pyridinylmethyl)indole (26)	200(5)	325(6)
3-(3-pyridinylmethyl)indole (33)	500(7)	500(=7)
indole	1250(=8)	500(=7)
indole-3-acetonitrile (6)	1250(=8)	1250+(9)
skatole*(25)	-	-

* The results for skatole were reported to be very poor but no data sent.

From the above results at least two conclusions can be drawn: firstly that the inactivity of indole and skatole (25) suggests that the properties of these compounds do not simply require the presence of just an indole or indole-3-methylene moiety; secondly that the compounds with an electron rich methylene substituent, ie 4-methoxyphenyl, 2,4,6-trimethylphenyl and 3-indolyl, were far more effective than those possessing electron deficient substituents, ie 2- and 3-pyridyl, and cyano. It was therefore hoped that an increase in the electron density on the benzene moiety would enhance the activity of these compounds still further. To test this possibility, 3-(4-hydroxybenzyl)indole (34), a new compound, and 3-(4-N,N-dimethylaminobenzyl)indole (35) were synthesised.



(34)

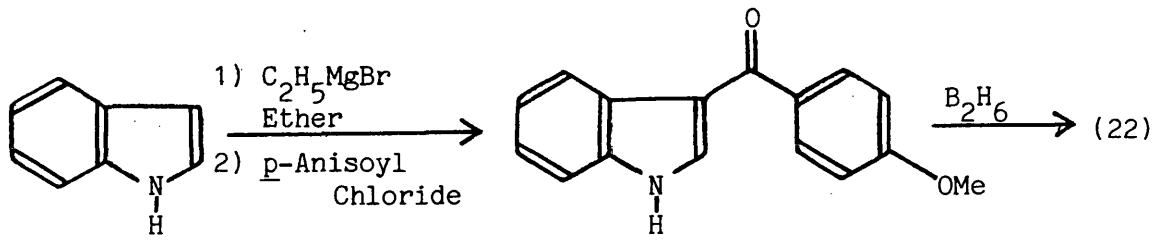


(35)

3-(4-N,N-Dimethylaminobenzyl)indole (35) was prepared by the same route as that used for the other benzylindole derivatives in excellent yield (83%). The starting materials were thus indole and the corresponding alcohol. 3-(4-Hydroxybenzyl)indole (34) was prepared by the demethylation of 3-(4-methoxybenzyl)indole (22) in reasonable yield (38%),

adopting a method reported by Minamikawa and Brossi[17] using trimethylsilyl iodide and quinoline.

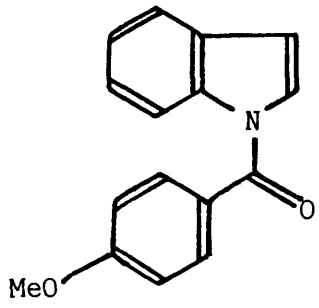
The 3-(4-methoxybenzyl)indole (22) used in this reaction was prepared from indole by an efficient two step synthesis via 3-(4-methoxybenzoyl)indole (36), reported by Jackson and Biswas[18] (Scheme 10).



(36)

SCHEME 10

The overall yield for this reaction was 72% compared to 32% for the original porcess[14]. It is interesting to note that when tetrahydrofuran was used instead of diethyl ether as solvent in the first step, 1-(4-methoxybenzyl)indole (37) was the major product.



(37)

Samples of 3-(4-hydroxybenzyl)indole (34) and 3-(4-N,N-dimethylaminobenzyl)indole (35) were sent for testing and both compounds proved to be extremely active in each of the two screens, their activity being of similar magnitude as butylated hydroxytoluene (BHT) (15) and α-tocopherol, two highly active inhibitors of chemical carcinogenesis (table 2).

TABLE 2

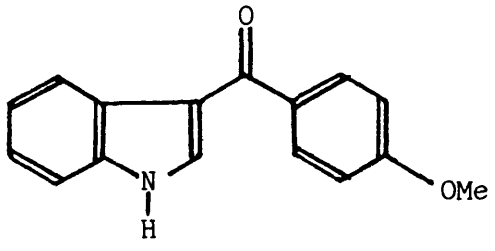
Biological data on compounds with increased electron density on the benzene moiety

Compounds	Oxidation system Conc 50% I μm (Ranking)	
	AIBN/CB	Fe/Asc
3-(4-hydroxybenzyl)indole (34)	13(1)	12(4)
3-(4-N,N-dimethylaminobenzyl)indole (35)	16(2)	1.5(2)
butylated hydroxytoluene (BHT) (15)	18(3)	1.2(1)
α-tocopherol	40(4)	10(3)

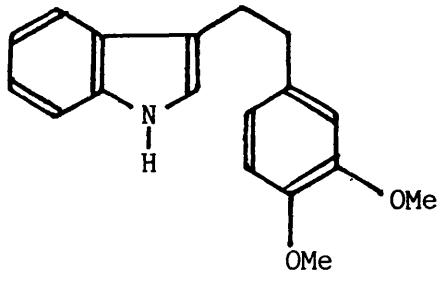
Next we examined the role of the methylene bridge. Our original criteria for synthesis of analogues of these dietary 3-indolylmethanes was the ability to form a stable radical, originally sited on the methylene bridge position, this radical being stabilized by both the methylene substituents. If this is in fact the case, then the prevention of this

process by oxidising the methylene bridge to the corresponding ketone, the insertion of another methylene into the bridge or the conversion of the bridging carbon into a quaternary centre should dramatically reduce the activity of the compound.

The first two of these assumptions could be tested by analysing the activity of 3-(4-methoxybenzoyl)indole(36), a compound previously synthesised en route to 3-(4-hydroxybenzyl)indole (34), and 3-[2-(3,4-dimethoxyphenyl)ethyl]indole (38) which has been previously synthesised in these laboratories for different purposes, and which was therefore also readily available.

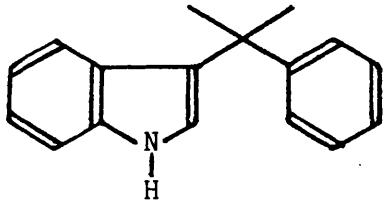


(36)



(38)

To test the third of these assumptions, a synthesis of the previously unknown 3-(1-methyl-1-phenylethyl)indole (39) was devised.

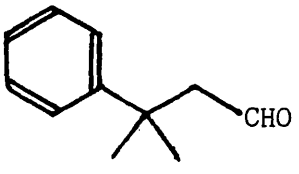


(39)

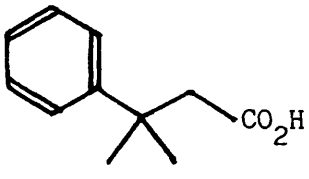
The synthesis of this compound (39) was not as straight-forward as might at first appear. It is complicated by the presence of a quaternary carbon at the site previously used for the final coupling reaction. Dimethylation of 3-benzoylindole was also ruled out due to the amidic character of the carbonyl function in this compound.

A Fischer indolisation[25,26], however, overcomes these problems allowing the troublesome quaternary carbon centre to be introduced before the final cyclisation process and away from the final coupling site.

The aldehyde required for the preparation of this target compound (39), namely 3-methyl-3-phenylbutanal (β-phenyl isovaleraldehyde) (40), is a known compound which has been prepared previously by Ruchardt[19] from the corresponding acid, 3-methyl-3-phenylbutanoic acid (41).

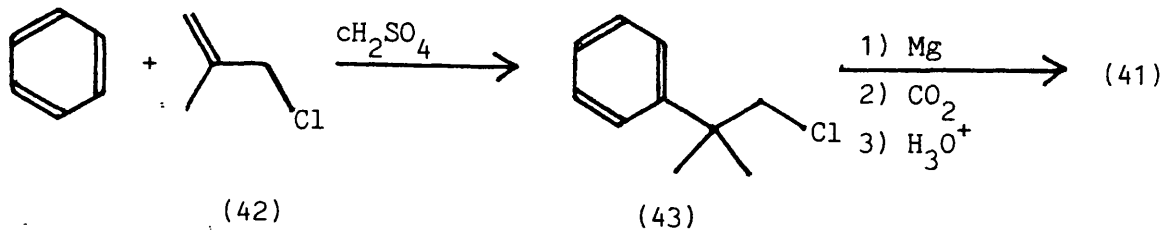


(40)



(41)

This acid (41) is synthesised in a two step process from benzene and 3-chloro-2-methylpropene (methallyl chloride) (42): an excess of benzene was reacted with the chloride (42), using concentrated sulphuric acid as catalyst, to yield neophyl chloride (43) which was subsequently reacted with magnesium and quenched with solid carbon dioxide, followed by dilute acid to yield the required acid (41) (Scheme 11).

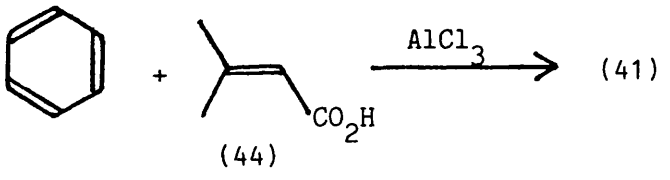


SCHEME 11

Ruchardt also synthesised other substituted benzene derivatives by this route[19].

In our hands, neophyl chloride (43) was prepared in 72% yield via the above route. However, even though the next stage in this process has been reported more than once[20,21] to be a viable route to the acid (41), we could not effect the reaction between neophyl chloride (43) and magnesium using ether as solvent, even with the addition of I_2, $HgCl_2$ and 1,2-dibromoethane as promoters. Slow reaction did occur, however, when tetrahydrofuran (THF) was used as solvent, but quenching the reaction mixture with solid carbon dioxide only afforded a 50% yield of the required acid (41).

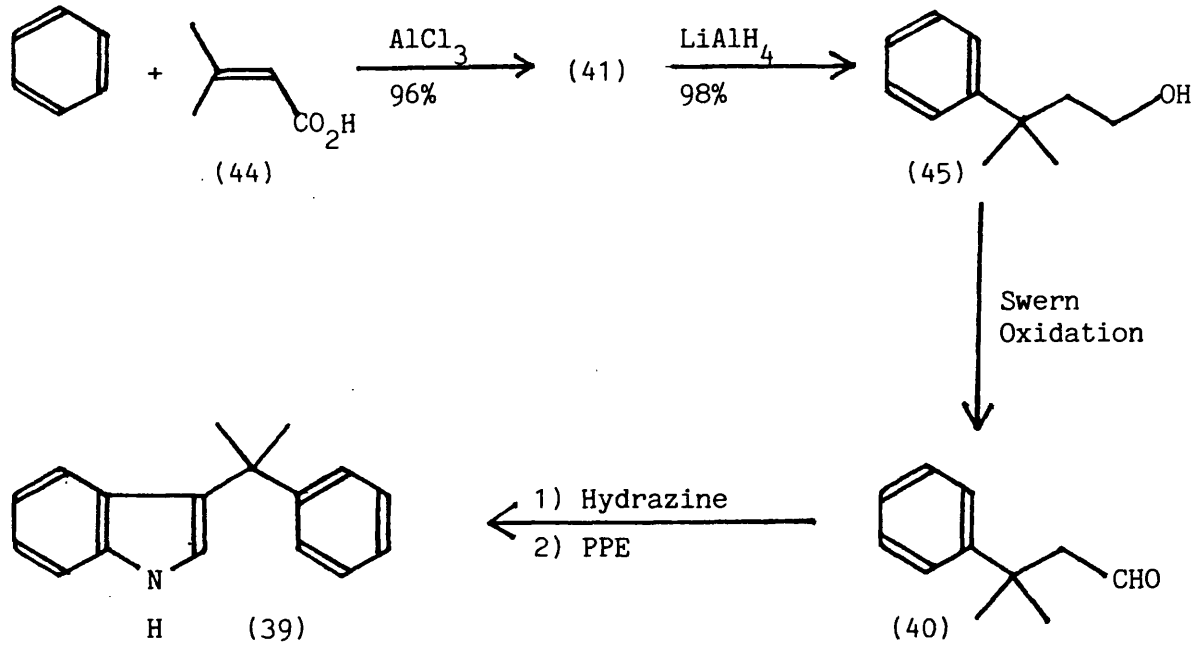
Fortunately 3-methyl-3-phenylbutanoic acid (41) has also been prepared[22] in a one step reaction, catalysed by aluminium(III) chloride, between benzene and 3-methylbut-2-enoic acid (β,β-dimethylacrylic acid) (44) (Scheme 12).



SCHEME 12

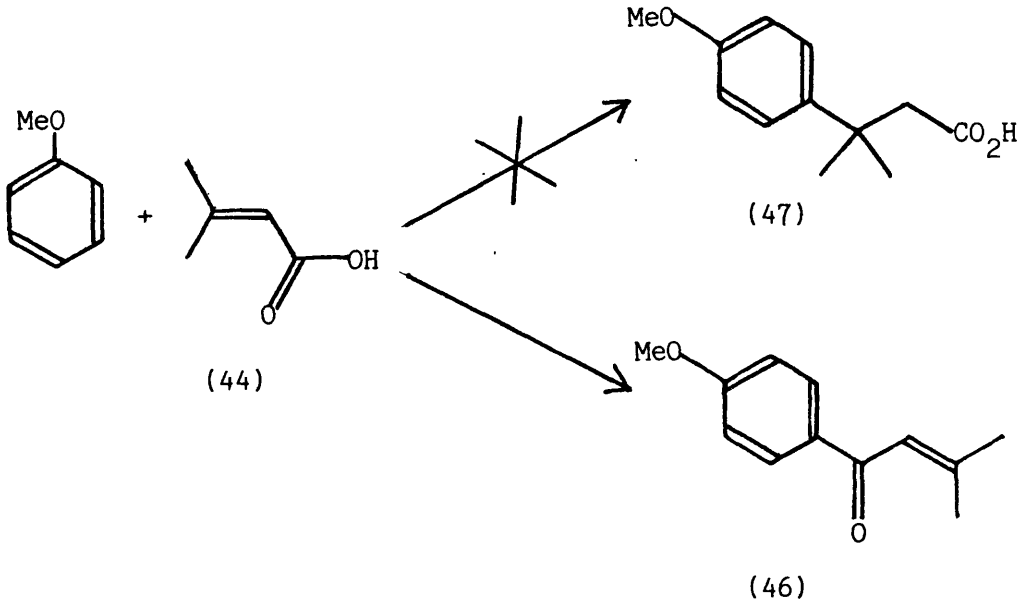
When tried, this reaction yielded the required acid (41) almost quantitatively. The acid (41) was reduced to alcohol (45) using lithium aluminium hydride and oxidised to the aldehyde (41) by a Swern oxidation[23,24] process. Both steps were again effectively quantitative.

Polyphosphate ester (PPE) was used to catalyse the final Fischer indole cyclisation as it has been reported[25] to give better yields of 3-substituted indoles from aldehydes than other potential catalysts. This final Fischer indolisation reaction yielded the required benzylindole (39) in 80% yield (Scheme 13).



SCHEME 13

It is interesting to note that, when anisole was used instead of benzene in the first step of the reaction, addition occurred at the acid moiety, and not at the terminal olefinic centre, as reported[22], to yield the ketone (47) (Scheme 14).



SCHEME 14

The reason for this may be that anisole is a slightly harder nucleophile than benzene and therefore attacks the harder of the two electrophilic centres of the α,β-unsaturated acid. Whatever the true explanation, a synthesis of the methoxylated acid was not pursued further, since we anticipated that the question posed over the biological activity of the disubstituted methylene group would be answered by an examination of the simple structure (39).

The three compounds prepared to examine our bridge-radical hypothesis were sent for testing, the results of which, together with data on some other relevant compounds, are given below. (Table 3)

TABLE 3

Biological data on the deactivated methylene bridge compounds and related compounds

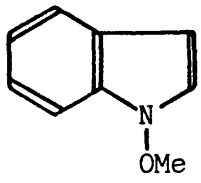
Compound	Oxidation System Conc.50% Iμm(Ranking)	
	AIBN/CB	Fe/Asc
3-[2-(3,4-dimethoxyphenyl)ethyl]indole (38)	115(2)	12(1)
3-(1-methyl-1-phenylethyl)indole (39)	80(1)	18(2)
3-(4-methoxybenzyl)indole (22)	300(4)	24(3)
3-benzylindole (21)	185(3)	36(4)
3-(4-methoxybenzoyl)indole (36)	- *	- *

* 250μm of (36) only caused a 10 - 20% drop in lipid peroxidation.

From these data, it can be seen that only in the case of the ketone (36) was the activity of the compound greatly affected by structural changes to the carbon bridge. In fact, in the case of the dimethyl derivative (39), the activity of the compound was actually enhanced relative to the parent compound (21).

Since it would seem that our original rationale for the activity of these compounds was misfounded, we had to look at other potentially "active sites" in these molecules and the known occurence of 1-hydroxyindole

derivatives in plants prompted an investigation of the potential role of the indole nitrogen. The first naturally occurring 1-hydroxyindole derivative, 1-methoxyindole (48), was isolated in 1962 from the Brassica species by Gmelin and Virtanen[27].



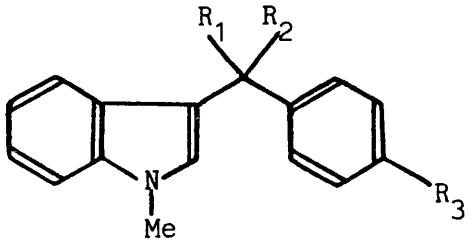
(48)

Since then, a few other 1-methoxyindoles have also been obtained from plant sources[28].

As it is known that N-oxidation is a key step in the metabolic activation of amine procarcinogen[29], it is feasible that a similar N-oxidation of indole derivatives may also occur, with the relatively unstable 1-hydroxyindole formed being O-methylated at a later stage in the compound's metabolism or on extraction from the plant. The inference is that the formation of this metabolite could be responsible for the activity of these compounds, both as a radical scavanger and by direct interaction with carcinogenic electrophiles, such as the diol epoxide of B[a]P (13).

To examine this possibility, three of the active compounds already prepared, namely 3-benzylindole (21), 3-(4-methoxybenzyl)indole (22) and 3-(1-methyl-1-phenylethyl)indole (39), were N-methylated with a view to preventing this N-oxidation process.

All three compounds were methylated using sodium hydride and methyl iodide and gave respectively 3-benzyl-1-methylindole (49), 3-(4-methoxy-benzyl)-1-methylindole (50) and 3-(1-methyl-1-phenylethyl)-1-methylindole (51) in good yields.



(49) R_1, R_2, R_3=H

(50) R_1, R_2=H, R_3=OMe

(51) R_1, R_2=Me, R_3=H

The biological results for these and their parent compounds are given in table 4. From these results it can be seen that N-methylat of the unsubstituted methylene compounds (21), (22), had only a slight, and in the case of (21) not necessarily detrimental, effect on their activities, whereas the activity of the substituted methylene bridge compound was completely destroyed. A possible explanation for this is that both the methylene bridge and the indole nitrogen are potential "active" sites in the molecule and therefore both must be blocked before the activity of the compound is destroyed. What this means in chemical terms is not yet clear, but will provide "food for thought" in future work.

These are, however, not the only potential oxidation sites in the molecule. It is known[30] for example that tryptamine, indole acetic acid and related compounds are oxidised by the liver to 6-hydroxyindole derivatives.

TABLE 4

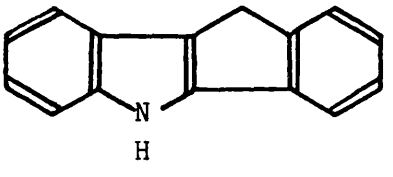
Biological data on N-methylated analogues and their parent compounds

Compound	Oxidation systems Conc. 50% I(μm)	
	AIBN/CB	Fe/Asc
3-benzylindole (21)	186	36
3-benzyl-1-methylindole (49)	500	13
3-(4-methoxybenzyl)indole (22)	300	24
3-(4-methoxybenzyl)-1-methylindole (50)	400	31
3-(1-methyl-1-phenylethyl)indole (39)	80	18
3-(1-methyl-1-phenylethyl)-1-methylindole (51)*	-	-

* Dosing with 1250 μm of (51) only caused 20 - 40% inhibition.

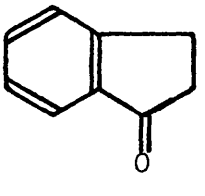
If oxidation does occur at the 1 or 6 position of the indole moiety, however, it would seem difficult to explain how the methylene substituent could radically affect the activity of these compounds, particularly in these in vitro experiments where biological transport is not a consideration. Oxidation of the benzyl derivative of our analogues also cannot be ruled out, especially since the activity of our compounds increases as the ease of oxidation of the benzyl derivative increases.

The situation was complicated still further when we examined the activity of a fused ring system where the methylene group was fixed into a tetracyclic ring system. The example chosen was 5,10-dihydroindeno[1,2-b]-indole (52).



(52)

5,10-Dihydroindeno[1,2-b]indole (52) was prepared from indan-1-one (53), using a Fischer indolisation process in 63% yield.



(53)

The tetracycle was sent for biological testing and to our amazement turned out to be the most active compound tested including the two standard antioxidants, BHT (15) and ∝-tocopherol. In fact, it was so active in the iron/ascorbic acid oxidation system that the minimum dose listed, namely 4 μm caused 90% peroxidation inhibition (Table 5)

TABLE 5

Biological data on 5,10-dihydroindeno[1,2-b]indole (52)

Compound	Oxidation Systems Conc. 50% I(μm)	
	AIBN/CB	Fe/Asc
5,10-dihydroindeno[1,2-b]indole (52)	14	*
BHT (15)	18	1.2
∝-tocopherol	40	10

* too low to be measured by this test system

The reason for the extraordinary activity of this tetracyclic compound (52) is completely unknown and would seem difficult to rationalise by simply considering primary oxidation sites.

From the experimental data already obtained it would seem that the activity of these compounds could not necessarily be pinned down to one site in the molecule. It was therefore considered important to try to

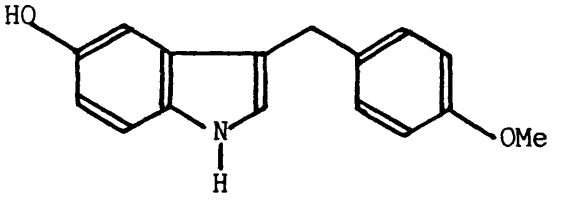
ascertain the position of the primary site of oxidation in our compounds by using an oxidation system that could mimic the oxidation of those compounds in the body.

The work carried out by Jepson et al[30] to ascertain the true structure of the oxidative metabolites of tryptamine and its analogues uses the actual liver microsomal system itself in an in vitro experiment by the removal and processing of fresh rabbits livers. This method, however, can only be operated on the 10^{-6} molar level and therefore could not be used if the potential oxidation product(s) were to be separated and fully characterised.

We therefore looked for an alternative, and hopefully chemical, oxidation system and, on searching the literature, found that the aqueous iron/ascorbic acid system, used to test our compound, had also been used on the 10^{-3} molar level on aromatic substrates to yield oxidation products in sufficient quantity to allow characterisation. Such products were adjudged to be identical to those formed in vivo.[31,32] In this system the substrate is shaken with EDTA, ascorbic acid and ferrous sulphate in a pH 6.7 phosphate buffer at 37°C under an oxygen atmosphere for two hours.

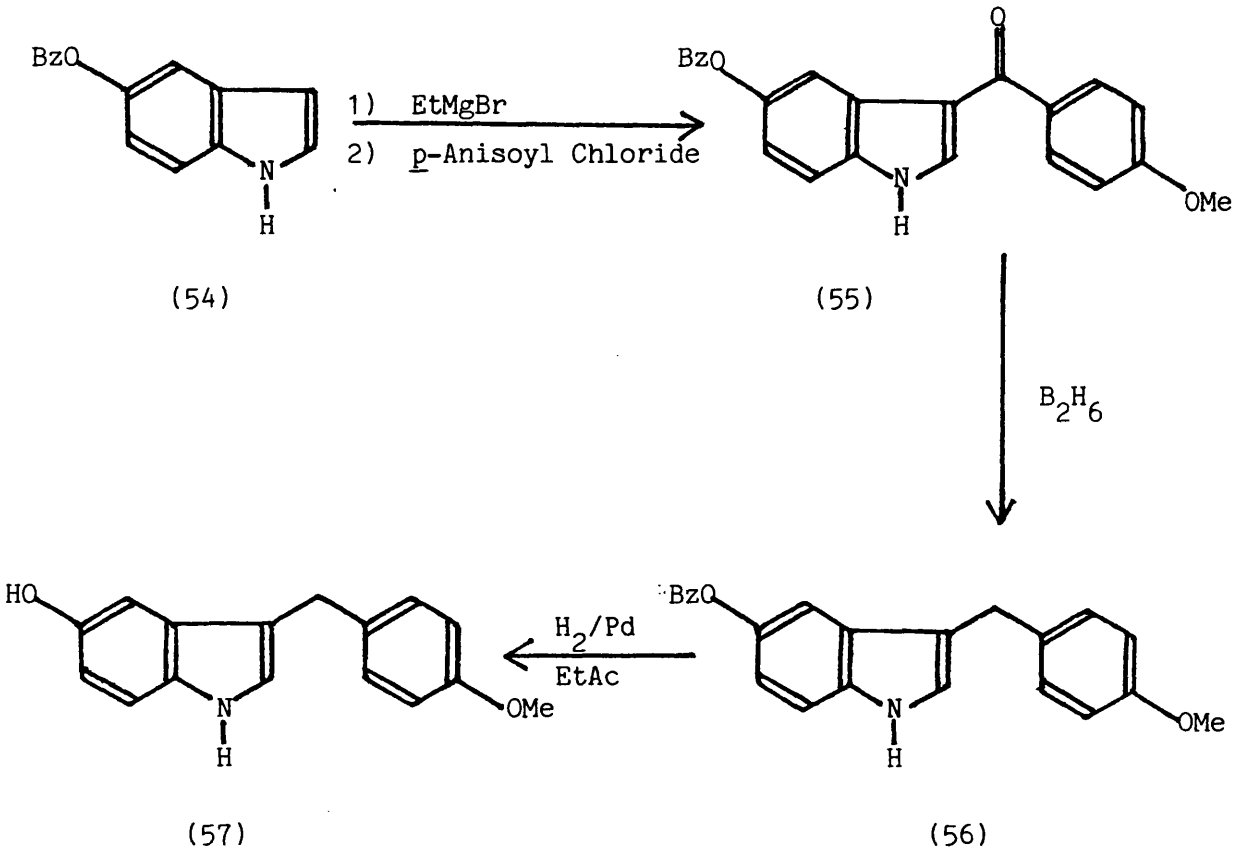
We attempted a similar experiment using 3-benzylindole (21) as a test subject. Unfortunately, no oxidation was observed and we concluded at first that this must be due to the insolubility of this compound in water. Next we added sufficient acetone to the medium to effect solution of our substrate but again quantitative recovery of the starting material was obtained at the end of the experiment. Finally a two phase system using dichloromethane and a phase transfer catalyst was tried but once again the treatment failed to generate any oxidation products. The failure of this oxidation system was a disappointment and left the question of the primary site of oxidation of these molecules still completely unanswered.

As the last study in this section, the effect of an electron donating substituent on the 5-position of the indole ring was examined. In other systems substitution at this site is frequently observed to increase biological effects, for example in the pyrido[4,3-b]carbazoles. Although the 5-substituted 3-(4-hydroxybenzyl)indole or 3-(4-N,N-dimethylaminobenzyl)indole derivative would probably be the most active compounds of this type, their original activity is so high that the true effect of this new substituent may not be seen. For this reason the 5-substituted 3-(4-methoxybenzyl)indole derivative, 5-hydroxy-3-(4-methoxybenzyl)indole (57), was selected as a target.



(57)

This new compound was made by the same route as for the parent compound (22) using 5-benzyloxyindole instead of indole in the first step. This was followed by diborane reduction of the intermediate ketone as before. Finally, the O-benzyl group was removed by hydrogenolysis to afford the required substrate (57). Yields throughout this sequence were acceptable (Scheme 14).



SCHEME 14

5-Hydroxy-3(4,methoxybenzyl)indole (57) has recently been sent for testing, but as yet no information has been received back in the United Kingdom.

CONCLUSION

In summary, we have indicated that certain 3-indolylmethanes act as very effective anti-oxidants and, by analogy with work on similar compounds, are probably valuable in the inhibition of cancer caused by exposure to polyaromatic hydrocarbons (see appendix 2). The mode of action of these indoles remains uncertain, although we have obtained some results which need to be investigated further. Future work will also consider the metabolic fate of these structures and this study will be complemented by some electrochemical oxidative experiments on the indoles to determine their fates in vitro. Anti-oxidants do, of course, have commercial value in many fields (as preservatives in food, for example) and toxicological assessments of our compounds are under way. Sadly none of them appear to be useful in the treatment of rheumatic diseases - or at least in the usual test systems used to predict this property.

EXPERIMENTAL

Melting points were recorded on an Electrothermal Mark II apparatus and are uncorrected. Infra-red spectra were recorded on Perkin-Elmer 197 or 1310 grating spectrophotometers, using polyethylene film as a reference standard. Ultra-violet spectra were recorded on a Perkin 402 instrument and in ethanol solution, unless otherwise stated. ^{1}H NMR spectra were run at 60MHz on Perkin-Elmer R24B and Varian EM360 spectrometers, and at 400Hz using the SERC facility at Warwick University. Mass spectra and high resolution accurate mass measurements were determined on a VG7070E instrument with a VG2000 data system.

TLC analysis was performed on Merck DC-Alufolien plates coated with Kieselgel 60 F_{254}. Unless otherwise stated, column chromatography was carried out using short path columns packed with Merck 7747 silica gel, the solvent being eluted under pressure provided by hand bellows. All solvents used for chromatography were distilled prior to use. The petroleum ether used in all experiments was the 60-80°C boiling fraction.

All other general reagents and solvents were purified when required using the methods described by Perrin et al[39].

The sodium hydride used in all experiments was a 60% suspension in oil, the oil being removed prior to use with petroleum ether.

In all experiments the excess solvent was removed under reduced pressure to avoid any unnecessary heating.

All yields quoted are uncorrected, unless otherwise stated.

The Dietary Indoles

Indole-3-carbinol (8)

Indole (15g; 0.13mol) in acetic anhydride ($25cm^3$) was added dropwise, over a period of 33 minutes, to a stirred solution of imidazole (8.7g; 0.13mol) in acetic anhydride ($50cm^3$) at 125°C. After complete addition the solution was stirred at this temperature for a further 30 minutes, then the acetic anhydride was removed under reduced pressure affording a yellow solid which was crystallised from acetonitrile to yield 1,3-diacetyl-2-(3-indolyl)-4-imidazoline (17) (26.4g; 77%) m.p. 215-216°C (lit.[11], 215-216°C).

The imidazoline (17) (25g; 0.1mol) was added to a solution of sodium hydroxide (12.5g; 0.3mol) in 95% ethanol ($250cm^3$) and distilled water ($125cm^3$) and the mixture heated to reflux with TLC monitoring. After one hour the reaction was seen to have gone to completion, so the now dark orange solution was poured onto distilled water ($1250cm^3$), then neutralised by the careful addition of concentrated hydrochloric acid. The resultant solid precipitate was filtered and crystallised from absolute ethanol to yield 3-formylindole (18) (9.3g; 69%) m.p. 196-198°C (lit.[11], 196-198°C).

I.R. - ν_{max} (Nujol) cm^{-1}; 3150(N-H), 1635(C=O)

N.M.R. - $\delta_H(CDCl_3)$ ppm; 11.66(1H,br.s,N-H), 9.90 (1H,s,CHO), 8.22-8.10(1H,m), 7.90(1H,s), 7.50-7.40(1H,m), 7.30-7.07(2H,m).

M.S. - (low eV,EI)m/z; 145(82%,[M^+]), 144(100%), 116(38%).

To 3-formylindole (18) (14g; 9.7mmol) dissolved in methanol ($50cm^3$) and stirred in an ice bath was added an excess of sodium borohydride in approximately 100mg aliquots. After this addition the reaction mixture

was stirred at room temperature for 30 minutes, then poured onto an ice/water mixture and carefully saturated with potassium carbonate, before extraction with diethyl ether. Removal of the solvent yielded an off-white solid which was crystallised from toluene to yield indole-3-carbinol (8) (1.2g; 85%) m.p. 97-98°C (lit.[33], 99-100°C).

U.V. - λ_{max}nm; 222.

I.R. - ν_{max} (Nujol) cm^{-1}; 3385(N-H), 3385-3140(O-H).

M.S. - (low eV, EI)m/z; 147(57%[M^+]), 130(100%), 129(95%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 7.80-7.50(1H,m), 7.20-6.90(4H,m), 6.50(1H,s), 5.33(2H,s,$\underline{CH_2}$), 2.92(1H,br.s,$\underline{OH}$).

- $\delta_C(CDCl_3)$ppm; 135.5(s), 129.1(s), 127.4(d), 122.1(s), 121.0(d), 120.2(d), 119.1(d), 102.0(d), 69.0(t,$\underline{CH_2}OH$).

3,3'-Diindolylmethane (7)

1) Condensation of indole with glyoxylic acid and decarboxylation

To indole (17.5g; 0.15mol) vigorously stirred in water (230cm^3) at 50-55°C was added a solution of glyoxylic acid (19) (16.3g; 0.22mol) in water (150cm^3) in a dropwise manner. After the addition, the mixture was stirred for one hour, then the purple precipitate formed was filtered off and crystallised from ethanol/water to yield 3,3-diindolylacetic acid (20) (21.2g; 97%) m.p. 188-192°C (lit.[12], 192°C).

I.R. - ν_{max} (Nujol) cm^{-1}; 3400(N-H), 1700(C=O).

N.M.R. - $\delta_H(d^6DMSO)$ppm; 10.66(2H,s,N-$\underline{H}$), 7.6-6.8(10H,m,aromatic protons), 5.3(1H,s,$\underline{CH}CO_2H$).

3,3'-Diindolylacetic acid (20) (1g; 3,4mmol) was heated at 195°C for 30 minutes and then cooled to room temperature. TLC examination of the resultant solid showed a multiproduct mixture. All attempts to purify this mixture by boiling with activiated charcoal, column chromatography and cystallisation failed.

The reaction was repeated several times with the same end result.

2) Condensation of indole with formaldehyde

To a solution of indole (4.75g; 0.4mol) in water ($200cm^3$) was added a solution of formalin (0.2mol). The reaction mixture was heated in the dark at 75-80°C for five and a half hours, then allowed to cool overnight. The resultant white precipitate was filtered, washed with water and recrystallised from methanol to yield 3,3-diindolylmethane (7) (4g; 81%) m.p. 164-165°C (lit.[13], 168°C).

U.V. - λ_{max}nm; 225.

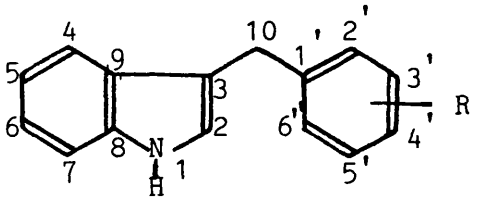
I.R. - ν_{max} (Nujol); 3395(N-H).

M.S. - (low eV,EI)m/z; 246[M^+].

N.M.R. - δ_H($(CD_3)_2CO$)ppm; 9.30(2H,br.s.,exchanged by deuteration,N-H), 7.50-6.70(10H,m,aromatic protons), 4.1(2H,s,CH_2)

- δ_C(d^6-DMSO)ppm; 136.4(s), 127.2(s), 122.7(s), 120.7(d), 118.6(d), 118.0(d), 114.2(d), 111.3(d), 21.0(t,CH_2).

Analogues of Dietary Indoles



Disproportionative condensation of indole with benzyl alcohols

Standard procedure[16]:-

To a solution of the alcohol (0.1mol) in p-cymene (44cm^3) was added crushed potassium hydroxide (900mg; 0.04mol) and the mixture was then heated at reflux using a Dean-Stark trap for about one hour. The reaction mixture was then cooled to allow the addition of indole (6g; 0.05mol) and nickel (250mg; 0.04mol). Heating at reflux was then recommenced. In the literature[16] the reaction was followed by evolution of water measured in the Dean-Stark trap. However, in our hands, this method was found to be unreliable and so the reactions were monitored by TLC. If the reaction was not normally complete after 4 hours, it was invariably left on overnight (15 hours).

According to the literature[16], after the reaction had gone to completion, simply cooling the resultant mixture afforded the product. In our hands this did not always occur and so the product was isolated and purified by bulb to bulb distillation.

3-Benzylindole (21)

Benzyl alcohol ($10cm^3$; 0.1mol) and indole (6g; 0.05mol) were condensed using the standard procedure and a reaction time of 15 hours. Cooling afforded an off-white precipitate which was filtered, washed with water ($2 \times 25cm^3$) and recrystallised from toluene to yield 3-benzylindole (21) (8g; 75%) m.p. 105-107°C (lit.[14], 105-106°C).

U.V. - λ_{max}nm(ε); 282(4340), 227(9023).

I.R. - $\nu_{max}(Nujol)cm^{-1}$; 3400(N-H).

M.S. - (low eV,EI)m/z; 207[M^+].

- (70 eV,EI)m/z; 207(84%,[M^+]), 206 (70%), 130(100%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 7.50-7.32(2H,m), 7.30-6.90(8H,m+s), 6.65(1H,s), 4.00(2H,s,$\underline{CH_2}$).

- $\delta_C(CDCl_3)$ppm; 141.2(s,C-1'), 136.3(s,C-8), 128.7(d), 128.3(d), 127.4(s,C-9), 125.8(d), 122.3(d), 122.0(d), 119.3(d), 119.1(d), 115.6(s,C-3), 111.1(d,C-7), 31.5(t,$\underline{CH_2}$).

3-(4-Methoxybenzyl)indole (22)

The standard procedure was used but TLC analysis during the reaction showed several product spots. After 15 hours at reflux, there was no great change in the relative intensities of the various spots on TLC, so the reaction was stopped. Cooling did not afford any precipitate, so the p-cymene was removed under reduced pressure and the product purified by bulb to bulb distillation to yield 3-(4-methoxybenzyl)indole (22) (4g; 33%) b.p. 250°c/0.3mmHg, m.p. 82-84°C (lit.[14], 84-84.5°C).

U.V. - λ_{max}nm(ε); 280(8017), 229(13075).

I.R. - ν_{max}(Nujol)cm^{-1}; 3370(N-H).

M.S. - (low eV,EI); 237[M^+].

- (70 eV,EI); 237(100%,[M+]), 236(67%), 130(60%).

N.M.R. - δ_H(DMSO)ppm; 10.8(1H,br.s,exchanged by deuteration,N-H), 7.28-6.66(9H,m,aromatic protons), 3.96(2H,s,CH_2), 3.66(3H,s,OCH_3).

- δ_C(DMSO)ppm; 157.3(s,C-4'), 136.4(s,C-8), 133.6(s,C-1'), 129.9(d,C-2',6'), 127.0(s,C-9), 123.0(d), 120.8(d), 118.5(d), 118.1(d), 114.3(s), 113.5(d,C-3',5'), 111.3(d,C-7), 54.8(q,OCH_3), 30.1(t,CH_2).

3-(2-pyridinylmethyl)indole (26)

The standard procedure was again used with a reaction time of 15 hours. Cooling yielded a white solid which was crystallised from cyclohexane and chloroform to yield 3-(2-pyridinylmethyl)indole (26) (35g; 33%) m.p. 105-106°C (lit.[35],104°C).

U.V. - λ_{max}nm(ε); 270(7920), 225(11954).

I.R. - ν_{max}(Nujol)cm^{-1}; 3270(N-H).

M.S. - (low eV,EI)m/z; 208[M^+].

- (70eV,EI)m/z; 208(100%,[M^+], 207(43%), 130(91%).

N.M.R. - δ_H(d^6DMSO)ppm; 10.84(1H,br.s,exchanged by deuteration,N-H), 8.44(1H,d,J=3Hz,H-3'), 7.60-6.80(8H,m,aromatic protons), 4.20(2H,s,CH_2).

- δ_C(d^6DMSO)ppm; 160.2(s,C-1'), 150.6(d,C-3'), 138.7(d,C-4'), 138.2(s, C-8), 129.0(s,C-9), 125.3(s,C-3), 124.8(d), 123.3(d), 120.6(d), 114.4(d), 113.4(d), 36.0(t,CH_2).

3-(4-N,N-dimethylaminobenzyl)indole (35)

This reaction was carried out on a slightly smaller scale than normal, using 0.066 moles of the alcohol, and 0.033 moles of indole. The reaction was heated at reflux for 15 hours as before, then cooled to yield an off-white solid which was washed with water (2 x $50cm^3$) and recrystallised from ethanol to yield 3-(4-N,N-dimethylaminobenzyl)indole (35) (7.1g; 83%) m.p. 143 - 144°C (lit.[36], 143 - 144°C).

U.V. - λ_{max}nm(ε); 258(17277), 223(27125).

I.R. - ν_{max}(Nujol)cm^{-1}; 3160(br.,N-H).

M.S. - (low eV,EI)m/z; 250[M^+].

- (70 eV),EI)m/z; 250(100%,[M^+]), 249(76%), 130(22%).

- Accurate mass: $C_{17}H_{18}N_2$; Requires 250.1469

Found 250.1435

N.M.R. - δ_H(d^6DMSOppm); 10.70(1H,br.s.,exchanged by deuteration, N-H), 7.50-7.38(2H,m,indole protons), 7.10(5H,d(J=8H_z)half of A,A',B,B' system H-2',6', and m,three indole protons), 6.60(2H,d(J=8H_z)half of A,A',B,B' system C-3',5'), 4.91(2H,s,CH_2), 2.76(6H,s,$N(CH_3)_2$).

- δ_C(d^6DMSO)ppm; 149.1(s,C-4'), 138.0(s,C-8), 129.5(s,C-1'), 129.3(d), 127.7(s,C-9), 122.2(d), 121.8(d), 119.1(d), 116.4(s), 113.1(d,C-3', 5'), 111.0(d,C-7), 40.8(q,$N(CH_3)_2$), 30.5(t,CH_2).

Route 3-(2,4,6-trimethylbenzyl)indole (24)

a) 2,4,6-trimethylbenzyl alcohol (32)

To a solution of mesitylene (29) (63.6g; 0.53mol) in carbon tetrachloride ($50cm^3$) stirred in an ice bath was added a solution of bromine ($28.8cm^3$; 0.56mol) in carbon tetrachloride ($50cm^3$) at a rate that maintained the internal temperature between 10 - 15°C. The hydrogen bromide

gas evolved was passed into water. After the addition was completed, the ice bath was removed and the reaction mixture left to stand for 1 hour before being washed with water (2 x 50cm^3), then with 50% sodium hydroxide (2 x 50cm^3), dried ($CaCl_2$), filtered and evaporated. The residue was distilled under reduced pressure to yield bromomesitylene (30)(11.2g; 68%) at 105 - 107°C/16 - 17 mmHg. To a stirred suspension of magnesium (8.5g; 0.35mol) in dry diethyl ether (20cm^3) was added a solution of bromomesitylene (30) (20g; 0.1mol) and ethyl bromide (21.8g; 0.2mol) in dry diethyl ether (100cm^3). About 3cm^3 of the solution was initially added in one portion to initiate the reaction, followed by dropwise addition at a rate that maintained the reaction mixture at gently reflux. After addition, the reaction mixture was maintained at reflux for a further 30 minutes, by external heating, then cooled and the liquid decanted onto dry ice (60g) with manual stirring. When the bulk of the dry ice had evaporated, 20% hydrochloric acid (100cm^3) and diethyl ether (50cm^3) were added and the two phase system shaken thoroughly, then allowed to settle. The aqueous layer was discarded and the ethereal layer washed with cold water (3 x 50cm^3) and then extracted with 10% sodium hydroxide (50cm^3). The aqueous layer was neutralised using 20% hydrochloric acid to afford a white precipitate which was filtered, washed with water (2 x 50cm^3) and air dried to yield mesitoic acid (31) (14.6g; 90%) m.p. 142 - 147°C (lit.[16], 152 - 154°C).

I.R. - ν_{max}(Nujol)cm^{-1}; 3700 - 3100(br,OH), 1680(C=O).

M.S. - (low eV,EI)m/z; 164[M$^+$].

To a suspension of lithium aluminium hydride (4g; 0.1mol) in dry THF (75cm^3) stirred under a flow of nitrogen and cooled in an ice bath was added dropwise a solution of mesitoic acid (31) (7.9g; 48mmol) in dry THF

($75cm^3$). After the addition, the ice bath was removed and the reaction mixture heated to reflux for 90 minutes, then cooled and a solution of saturated sodium potassium tartrate ($10cm^3$) carefully added. The liquid was then decanted off and the solid residue washed with ethyl acetate (2 x $30cm^3$). The organic fractions were combined, washed with 2N sodium hydroxide (2 x $100cm^3$) and water (2 x $100cm^3$), then dried ($MgSO_4$), filtered and the solvent removed to yield 2,4,6-trimethylbenzyl alcohol (32) (4g; 56%) m.p. 85 - 87°C (lit.[34], 87 - 89°C).

I.R. - ν_{max}(Nujol)cm^{-1}; 3160(br,O-H), finger print region also matches literature[34].

M.S. - (low eV,EI)m/z; 150(100%,[M^+], 132(69%).

b) 3-(2,4,6-Trimethylbenzyl)indole (24)

2,4,6-Trimethylbenzyl alcohol (32) was condensed with indole on a smaller scale (2.5g of alcohol) than usual, using the standard procedure and a reaction time of 15 hours. No precipitate was produced on cooling, so the p-cymene was removed under reduced pressure. The resultant gum was then distilled under high vacuum, using a 'bulb to bulb' system to yield 3-(2,4,6-trimethylbenzyl)indole (24) (205mg; 10%), b.p. 250°C/0.5 - 0.7mmHg m.p. 134 - 134.5°C.

U.V. - λ_{max}nm(ε); 283(5691), 224(16885)

I.R. - ν_{max}($CHCl_3$)cm^{-1}; 3500(N-H).

M.S. - (low eV,EI)m/z; 249(100%,[M^+]), 130(82%).

N.M.R. - δ_H($CDCl_3$)ppm; 7.80 - 7.50(2H,m), 7.30 - 7.10(3H,m), 6.72(2H,s, C-3',5'), 6.35(1H,s), 3.90(2H,s,CH_2), 2.22(3H,s,p-CH_3), 2.18(6H,s,o-CH_3).

Attempted preparation of 3-(4-nitrobenzyl)indole (23)

The reaction was attempted under the standard conditions; however, on heating 4-nitrobenzyl alcohol (15g; 0.1mol) with potassium hydroxide (900mg; 0.04mol), a black precipitate steadily formed. Undeterred, indole (6g; 0.05mol) and nickel (250mg; 0.04mol) were added and the resultant mixture heated to reflux for 15 hours. The reaction was then cooled and filtered to yield 16.9g of a black solid, which was insoluble in all the common solvents including ethyl acetate acetone and methanol.

Route to 3-(4-hydroxybenzyl)indole (34)

a) 3-(4-Methoxybenzoyl)indole (36)

To a solution of indole (11.7g; 0.1mol) in dry diethyl ether ($50cm^3$) stirred in an ice-bath under a nitrogen atmosphere was added dropwise a solution of ethylmagnesium bromide (0.1mol) in dry diethyl ether ($30cm^3$). After the addition was completed, the ice-bath was removed and the reaction mixture heated to reflux for 30 minutes. The ice-bath was then replaced and a solution of p-anisoyl chloride ($13.6cm^3$; 0.1mol) in dry diethyl ether ($20cm^3$) added slowly to the reaction mixture, which was then heated to reflux for one hour. After this time the reaction mixture was cooled and then carefully added to a stirred solution of 10% ammonium chloride ($50cm^3$) and crushed ice. This yielded an orange solid, 3-(4-methoxybenzoyl)-indole (36) (20.3g; 91%) which was filtered and crystallised from ethanol to yield an oranges cystalline solid, m.p. 207 - 208°C (lit.[18], 207 - 208°C).

I.R. - ν_{max}(Nujol)cm^{-1}; 3175(br,NH), 1617(C=O).

M.S. - (low eV,EI)m/z; 251[M^+].

- (70 eV,EI)m/z; 251(84%,[M^+]), 144(100%).

N.M.R. - δ_H(d^6DMSO)ppm; 11.90(1H,br.s,exchanged by deuteration, N-H), 8.40 - 8.20(1H,m,H-4), 7.98(1H,s,H-2), 7.88(2H,d(J=8H_z)part of A,A',B,B' system, C-2',6'), 7.64 - 7.48(1H,m), 7.36 - 7.20(2H,m), 7.08(2H,d(J=8Hz)part of A,A',B,B' system,C-3',5'), 3.80(3H,s,O$\underline{CH_3}$).

b) 3-(4-Methoxybenzyl)indole (22)

To a solution of 3-(4-methoxybenzoyl)indole (36) (6.25g; 0.025mol) and sodium borohydride (2.12g; 56mmol) in dry diglyme (50cm^3) stirred in an ice-bath under a nitrogen atmosphere was slowly injected boron trifluoride etherate (10cm^3; 75mmol). After the addition, the ice-bath was removed and the reaction was allowed to warm to room temperature. TLC analysis showed the reaction to have gone to completion after three hours, so the ice-bath was replace and methanol (5cm^3) carefully injected to destroy any remaining diborane, boron trifluoride etherate or sodium borohydride. The solvent was then evaporated off under reduced pressure and the residue partitioned between diethyl ether (50cm^3) and water (50cm^3). The ether fraction was washed with saturated potassium bicarbonate (1 x 50cm^3), water (1 x 50cm^3) and brine (1 x 50cm^3), dried ($MgSO_4$) and evaporated to afford a yellow solid (22) (4.7g; 80%) which was recrystallised from toluene and petroleum ether to yield a colourless crystalline solid. (See previous synthesis for spectra).

c) 3-(4-Hydroxybenzyl)indole (34)

To a solution of 3-(4-methoxybenzyl)indole (22) (2g; 8.4mmol) in quinoline (20cm^3) stirred in an ice bath under a slow stream of nitrogen was injected trimethylsilyl iodide (3.6cm^3; 25mmol). The ice-bath was removed and the reaction heated to 180°C for two hours. After this time

the reaction mixture was cooled and poured on to ice cold 2N HCl ($50cm^3$) and extracted with diethyl ether (2 x $30cm^3$). The ether layers were combined and washed with 2N hydrochloric acid (4 x $50cm^3$), water (2 x $50cm^3$) and brine (1 x $50cm^3$), then dried ($MgSO_4$) and the solvent evaporated. TLC analysis of the resultant gum showed two products. The more polar of the two products was thought to be the required phenol (34) and the other its silyl ether, so the gum was dissolved in methanol and left to stand overnight. TLC analysis after this time still showed a small amount of the less polar compound to be present, so several drops of hydrogen chloride saturated diethyl ether was added to the methanol solution with stirring. After one hour TLC showed all the less polar compound to have disappeared; however, a large baseline (in dichloromethane as solvent) had appeared, which tended to suggest that the strong acid had destroyed some of the product. Because of this, it was decided to use a weaker acid if the reaction was to be repeated. The methanol was removed under reduced pressure and the resultant gum columned using a dichloromethane / petroleum ether solvent system to yield 3-(4-hydroxybenzyl)indole (34) (720mg; 38%), which was crystallised from a diethyl ether / petroleum ether mixture, m.p. 142 - 143°C.

Analysis - $C_{17}H_{13}NO$; Requires C,80.69; H,5.87; N,6.27.
Found C,80.27; H,5.95; N,6.50.

U.V. - $\lambda_{max}^{nm}(\varepsilon)$; 281(9422), 224(30640).

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 3495(sh,N-H), 3300(br,O-H).

M.S. - (low eV,EI)m/z; 223[M^+].

- (70 eV,EI)m/z; 223(98%,[M^+]), 222(95%), 130(100%).

- Accurate mass: $C_{16}H_{13}NO$; Requires 223.0995
Found 223.0969.

N.M.R. - $\delta_H((CD_3)_2CO)$ppm; 9.85(1H,br.s,slight loss on deuteration,N-H), 8.12(1H,br.s,exchanged by deuteration,O-H), 7.52 - 7.28(2H,m, indole protons), 7.18 - 6.91(5H,m,H-2',6' + indole protons), 6.72(2H,d(J=9H_z),part of A,A',B,B' system,H-3',5'), 3.96(2H,s,CH_2).

- $\delta_C((CD_3)_2CO)$ppm; 156.2(s,C-4'), 137.8(s,C-8), 133.2(s,C-1'), 130.2(d,C-2',5'), 128.0(s,C-9), 123.4(d), 121.9(d), 119.6(d), 119.2(d), 115.9(s), 115.8(d,C-3',6'), 111.2(d,C-7), 31.2(t,CH_2).

Routes to 3-(1-methyl-1-phenylethyl)indole (39)

a) 3-Methyl-3-phenylbutanoic acid (41)

i) Via neophyl chloride (43):- To a stirred mixture of dry benzene ($60cm^3$) and concentrated sulphuric acid ($2cm^3$; d,1.84) was added freshly distilled 3-chloro-2-methylpropene (42) (18.2g; 0.2mol) over a period of two hours. The reaction was left stirring for a further 20 hours, then the benzene layer was decanted off, washed with water (4 x $50cm^3$) and dried ($MgSO_4$). The benzene was then removed and the residue distilled under reduced pressure to yield a colourless oil (43) (24.5g; 97%) b.p. 62°C/3mmHg (lit.[14], 51°C/1mmHg).

N.M.R. - $\delta_H(CDCl_3)$ppm; 7.13(5H,s,C_6H_5), 3.53(2H,s,CH_2Cl), 1.36(6H,s, CH_3).

A solution of neophyl chloride (43) ($9.8cm^3$; 0.06mol) in dry diethyl ether ($20cm^3$) was added dropwise to a stirred suspension of magnesium turnings (1.46g; 0.06mol) also in dry diethyl ether ($10cm^3$) under a nitrogen atmosphere. The vigorous reaction expected did not occur, so the addition was stopped and the reaction mixture gently

heated at reflux for 20 minutes. There was still no apparent reaction, so addition was continued and when complete the reaction mixture was again heated to reflux for 20 minutes. This also having failed to initiate the reaction, various catalysts, namely iodine, 1,2-dibromo-ethane and mercuric chloride were also tried but without success.

The reaction was repeated with even greater care taken to ensure that all the reagents were pure and dry, but still the reaction refused to start. A change of solvent was therefore considered.

The reaction was repeated using dry THF as solvent on a tenth of the previous scale. Magnesium (146mg; 6mmol), neophylchloride (43) ($0.98cm^3$; 6mmol) and a trace of iodine were heated to reflux, under a nitrogen atmosphere, in dry THF ($20cm^3$) for three hours, then allowed to stir overnight (15 hours). On returning, it was apparent that some of the magnesium had reacted, so the reaction mixture was decanted onto dry ice. When most of the dry ice had evaporated, 2N hydrochloride acid ($20cm^3$) was added and the mixture stirred for ten minutes. The two layers were then separated. The acid layer was washed with ethyl acetate (2 x $50cm^3$), then discarded. The organic fractions were combined, washed with water (2 x $75cm^3$), dried ($MgSO_4$), filtered and the solvent removed to yield a yellow gum (1g). NMR analysis showed this to be a 50:50 mixture of the starting chloride (43) and the required acid (41).

ii) Direct from β,β-dimethylacrylic acid (44):- Dry benzene ($50cm^3$) and β,β-dimethylacrylic acid (44) (2g; 20mmol) were heated to reflux in a Dean-Stark trap to remove any final traces of water present. The mixture was then cooled in an ice-bath and aliquots of aluminium

chloride (5g in total; 37mmol) were added over 20 minutes. When all the aluminium chloride had been added, the ice-bath was removed and the reaction mixture stirred at room temperature, and regular TLC analyses taken. After four hours, the reaction was seen to have gone to completion, so the mixture was carefully poured onto ice cold 2N hydrochloric acid ($50cm^3$) and stirred for 20 minutes. Ethyl acetate ($50cm^3$) was then added and the two phase system thoroughly shaken. The organic layer was then separated, washed with water ($2 \times 50cm^3$), dried ($MgSO_4$) and the solvent removed to yield a yellow gum which, when added to ice, yielded a colourless solid (41) (3.6g; 100%), recrystallised from petroleum ether, m.p. 57 - 59°C (lit.[20], 58 - 59.5°C).

I.R. - ν_{max}(Nujol)cm^{-1}; 1700(C=O).

M.S. - (low eV,EI)m/z; 178(41%,[M^+]), 119(100%).

N.M.R. - δ_H($CDCl_3$)ppm; 10.75(1H,br.s,exchanged by deuteration, O-H), 7.18(5H,s,$C_6\underline{H}_5$-), 2.60(2H,s,$\underline{CH}_2$), 1.50(6H,s,$\underline{CH}_3$).

- δ_C($CDCl_3$)ppm; 178.1(s,$\underline{CO}_2H$), 148.0(s,C-1'), 128.2(d), 126.1(d), 125.4(d), 48.1(t,$\underline{CH}_2CO_2H$), 37.0(s,>$\underline{C}(CH_3)_2$), 28.8(q>C($\underline{CH}_3$)$_2$).

b) 3-Methyl-3-phenylbutanol (45)

To a stirred suspension of lithium aluminium hydride (1.25g; 33mmol) in dry diethyl ether ($20cm^3$) in an ice bath and under a nitrogen atmosphere was slowly added a solution of the acid (41) (2.5g; 14mmol) in dry diethyl ether ($10cm^3$). On completion of the addition, the ice bath was removed and the reaction mixture left to stir for 15 hours. After this time TLC analysis of the reaction mixture showed it to have gone to completion so

the ice bath was then replaced and a saturated solution of sodium potassium tartrate ($5cm^3$) carefully added. The two layers were then separated. The aqueous layer was washed with diethyl ether (2 x $40cm^3$), then discarded. The organic fractions were then combined, washed with water (2 x $50cm^3$), dried ($MgSO_4$), filtered and evaporated to yield a yellow oil (45) (2.3g; 100%).

I.R. - ν_{max}(thin film)cm^{-1}; 3500-3100(br,O-H).

M.S. - (low eV,EI)m/z; 164(33%,[M^+]), 119(100%).

N.M.R. - δ_H($CDCl_3$)ppm; 7.13(5H,s,$C_6\underline{H}_5$), 3.70(2H,t(J=7H_z)-$CH_2\underline{CH}_2OH$), 1.87(2H,t(J=7H_z),-$\underline{CH}_2CH_2OH$), 1.62(1H,s,exchanged by deuteration, $O\underline{H}$), 1.30(6H,s,-$C\underline{H}_3$).

- δ_C($CDCl_3$)ppm; 148.8(s,C-1'), 128.3(d), 125.7(d), 125.6(d), 59.8(t), 46.8(t), 36.6(s,>$\underline{C}(CH_3)_2$), 29.3(q,>$C(\underline{CH}_3)_2$).

c) 3-Methyl-3-phenylbutanal (40)

To a stirred solution of freshly distilled oxalyl chloride ($1cm^3$; 11mol) in dichloromethane ($25cm^3$) at -60°C was slowly added a solution of dimethyl sulphoxide ($1.7cm^3$; 22mmol) in dichloromethane ($5cm^3$). After addition was completed, the reaction mixture was stirred for two minutes before the dropwise addition of a solution of the alcohol (45) (1.64g; 10mmol) in dichloromethane ($10cm^3$). The reaction was stirred for 20 minutes, then triethylamine ($7cm^3$) was slowly added. After a further five minutes stirring, the cooling bath was removed and the reaction allowed to warm to room temperature over one hour. Water ($50cm^3$) was then carefully added to the reaction mixture with stirring. The two layers were then separated, with the aqueous layer being washed with dichloromethane (2 x $25cm^3$), then discarded. The organic fractions were

combined, washed with water (2 x 50cm^3), dried ($MgSO_4$), evaporated and the residue purified by column chromatography to yield a colourless oil (40) (1.6g; 99%).

I.R. - ν_{max}(thin film)cm^{-1}; 2825, 2725(weak, CH aldehyde), 1725(C=O).

N.M.R. - δ_H($CDCl_3$)ppm; 9.24(1H,t(J=3Hz),C<u>H</u>O), 7.12(5H,s,C_6<u>H</u>$_5$-), 2.59(2H,d(J=3Hz),-C<u>H</u>$_2$CHO), 1.42(6H,s,C<u>H</u>$_3$).

- δ_C($CDCl_3$)ppm; 202.7(s,-<u>C</u>HO), 147.4(s,C-1'), 128.5(d), 126.3(d), 125.5(d), 56.5(t,<u>C</u>H$_2$CHO), 36.7(s,<u>C</u>(CH$_3$)$_2$), 29.4(q,C(<u>C</u>H$_3$)$_2$).

d) <u>3-(1-Methyl-1-phenylethyl)indole (39)</u>

The aldehyde (40) (4 90mg; 3mmol), phenylhydrazine hydrochloride (460mg; 3mmol) and absolute ethanol (10cm^3) were stirred under a nitrogen atmosphere at room temperature and triethylamine (420µl; 3mmol) slowly injected. As addition proceeded, so the hydrazine hydrochloride dissolved. After addition was completed, the reaction was stirred for a further 20 minutes before the solvent was removed and replaced by a solution of polyphosphonate ester (PPE) in chloroform (16cm^3; 20% w/v stock solution, 5 equivalents). The resultant orange solution was refluxed for five minutes, during which time it steadily turned green. The solvent was removed and the residue stirred with water (30cm^3) for one hour, then extracted with diethyl ether (3 x 20cm^3). The ethereal fractions were combined, washed with water (2 x 50cm^3), dried ($MgSO_4$) and evaporated. The resultant gum was purified by column chromatography to yield a light yellow gum(39)(560mg; 80%). All attempts to crystallise this compound failed.

Analysis - $C_{17}H_{17}N$: Requires; C,86.76; H,7.28; N,5.95%.

Found; C,86.71; H,7.08; N,5.88%.

I.R. - ν_{max}(thin film)cm^{-1}; 3420(sh,N-H).

M.S. - (low eV,EI)m/z; 235(100%,[M^+]), 220(31%).

- (70 eV,EI)m/z; 235(33%,[M^+]), 220(100%), 119(64%).

- Accurate mass: $C_{17}H_{17}N$; Requires - 235.1361

Found - 235.1339

N.M.R. - $\delta_H(CDCl_3)$ppm;7.57(1H,br.s,exchanged by deuteration, N-H), 7.36-6.92(10H,m,aromatic protons), 1.68(6H,s,$C(\underline{CH_3})_2$).

- $\delta_C(CDCl_3)$ppm; 149.9(s,C-1'), 137.1(s,C-8), 127.9(d), 126.3(d), 125.9(s), 125.7(s), 125.5(d), 121.5(d), 121.2(d), 120.6(d), 118.8(d), 111.1(d,C-7), 39.9(s,$>\underline{C}(CH_3)_2$), 30.6(q,$>C(\underline{CH_3})_2$).

Attempted synthesis of 3-(4-methoxyphenyl)-3-methylbutanoic acid (47)

To a solution of β,β-dimethylacrylic acid (44) (2g; 20mmol) in freshly distilled anisole (25cm^3), stirred in an ice bath, was added aluminium chloride (5g in total; 37 mmol) in aliquots (200mg) over a 20 minute period. When the addition was completed, the ice bath was removed and the mixture stirred at room temperature with regular TLC analyses taken. After 15 hours, the reaction was seen to have gone to completion, so the mixture was poured onto 2N hydrochloric acid (50cm^3) and stirred for 20 minutes. The two layers were then separated with the aqueous fraction being extracted with diethyl ether (2 x 30cm^3), then discarded. The organic fractions were combined, washed with water (2 x 50cm^3), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The resultant gum was purified using column chromatography to yield a colourless solid (3.3g) which was recrystallised from petroleum ether m.p. 28 - 28.5°C. From the spectra data, it was quite apparent that

1,2-and not 1,4-addition of anisole had occurred to yield the α,β-unsaturated ketone (46) (87%).

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 1640(C=O),1590(C=C).

M.S. - (low eV,EI)m/z; 190(61%,[M^+]), 175(100%), 159(45%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 7.90(2H,d(J=9Hz), aromatic protons ortho to ketone moiety), 6.88(2H,d(J=9Hz), aromatic protons ortho to methoxy group), 6.66(1H,s,olefinic proton), 3.80(3H,s,OCH_3), 2.16(3H,s, olefinic methyl cis to carbonyl), 1.96(3H,s,olefinic methyl trans to carbonyl).

- $\delta_C(CDCl_3)$ppm; 190.3(s,C=O), 163.0(s,aromatic carbon next to methoxy group), 155.0(s,), 132.5(s, aromatic carbon next to carbonyl), 130.5(d,aromatic carbons ortho to carbonyl), 121.2(d,), 113.7(d,aromatic carbons ortho to methoxy group), 55.4(q, CH_3O-), 27.8(q,olefinic methyl cis to carbonyl), 21.0(q,olefinic methyl, trans to carbonyl group).

5,10-Dihydroindeno[1,2-b]indole (52)

To a stirred solution of 1-indanone (53) (2.7g; 20mmol) and phenyl hydrazine hydrochloride (3g; 22mmol) in absolute ethanol (40cm^3) was slowly injected triethylamine (2.8cm^3, 22mmol). The hydrochloride suspension dissolved during the addition. After stirring for 20 minutes, the solvent was removed under reduce pressure and replaced by a solution of polyphosphonate ester (PPE) (5eq) in chloroform (200cm^3). The resultant solution was then heated to reflux for 20 minutes before the solvent was removed and the residue stirred with water (50cm^3) for one hour. This aqueous suspension was then extracted with diethyl ether (3 x 50cm^3) and the ethereal fractions combined, washed with water (2 x 50cm^3), dried ($MgSO_4$), filtered

and the solvent removed to yield 5,10-dihydroindeno[1,2-b]indole (52) (3g; 63%), recrystallised diethyl ether/petroleum ether m.p. 258 - 259°C (lit.[37], 284°C).

U.V. - λ_{max}nm(ε); 327(19364), 312(23635), 246(23200), 210(23916).

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 3460(sh,N-H).

M.S. - (low eV,EI)m/z; 205[M^+].

- (70 eV,EI)m/z; 205(100%,[M^+]), 204(66%).

N.M.R. - δ_H(DMSO)ppm; 11.48(1H,s,N-H), 7.70-6.90(8H,m,aromatic protons), 3.64(2H,s,CH_2).

- δ_C(DMSO)ppm; 147.4(s), 143.6(s), 140.7(s), 135.2(s), 126.6(d), 125.4(d), 124.5(d), 124.2(s), 120.9(d), 119.8(s), 119.2(d), 118.5(d), 117.7(d), 112.4(d), 29.8(t,CH_2).

N-Methylated compounds

General procedure

To a suspension of sodium hydride in dry THF stirred under nitrogen was slowly added the indole (1eq) as a solution in dry THF. After all the sodium hydride had reacted, the mixture was cooled in an ice bath and methyl iodide (1.1eq) slowly added, neat, via a syringe. The ice bath was removed and stirring continued for a further hour. The products were then purified by chromatography.

1) 3-Benzyl-1-methylindole (49)

3-Benzylindole (21) was methylated using this process to yield 3-benzyl-1-methylindole (49) in 93%. Crystallised petroleum ether, m.p. 59-60°C.

I.R. - ν_{max}(melt); No N-H peak.

M.S. - (low eV, EI)m/z; 221[M^+].

- (70 eV, EI)m/z; 221(90%,[M^+]), 220(56%), 144(100%).

N.M.R. - δ_H($(CD_3)_2CO$)ppm; 7.44(1H,d(J=7Hz), 7.30-6.82(8H,m), 6.73(1H,s), 3.97(2H,s,$C\underline{H}_2$), 3.49(3H,s,N-$C\underline{H}_3$).

- δ_C($(CD_3)_2CO$)ppm; 142.4(s,C-1'), 138.0(s,C-8), 129.2(d), 128.8(d), 128.6(s,C-9), 127.9(d), 126.3(d), 121.9(d), 119.6(d), 119.1(d), 114.5(s,C-3), 109.8(d,C-7), 32.3(t,$C\underline{H}_2$), 31.9(1,N-$C\underline{H}_3$).

2) 3-(4-Methoxybenzyl)-1-methylindole (50)

The target compound (50) was prepared by the above method from 3-(4-methoxybenzyl)indole (22) to yield an orange gum (50) (98%) after columning. All attempts to crystallise this compound failed.

I.R. - ν_{max}(Neat)cm^{-1}; No N-H.

M.S. - (low eV, EI)m/z; 251[M^+].

- (70 eV, EI)m/z; 251(82%,[M^+]), 250(62%), 144(100%).

- Accurate mass: $C_{16}H_{17}NO$; Requires - 251.1309

Found - 251.1281

N.M.R.- δ_H($CDCl_3$)ppm; 7.46(1H,m), 7.20-6.90(5H,m), 6.74(2H,d(J=9Hz) part of A,A',B,B' system, H-3',5'), 6.57(1H,s), 3.96(2H,s,$C\underline{H}_2$), 3.64(3H,s,O$C\underline{H}_3$), 3.50(3H,s,N$C\underline{H}_3$).

δ_C($CDCl_3$)ppm; 157.9(s,C-4'), 137.2(s,C-8), 133.5(s,C-1'), 129.5(d,C-2',6'), 126.9(s,C-9), 121.5(d), 119.2(d), 118.8(d), 114.7(s,C-3), 113.7(d,C-3',5'), 109.0(d,C-7), 55.0(1,O$C\underline{H}_3$), 32.3(q,N-$C\underline{H}_3$), 30.6(t,$C\underline{H}_2$).

3) 1-Methyl-3-(1-methyl-1-phenylethyl)indole (51)

The required compound (51) was prepared using the standard procedure

except that the compound was purified by bulb to bulb distillation to yield a white solid (98%) at 220°C/0.05mmHg recrystallised petroleum ether, m.p. 77 - 78°C.

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; No N-H absorption.

M.S. - (low eV,EI)m/z; 249(39%,[M^+], 234(100%).

- Accurate mass: $C_{18}H_{19}N$; Requires - 249.1517

Found - 249.1515

N.M.R. - $\delta_H(CDCl_3)$; 7.40-6.98(9H,m,aromatic protons), 6.85(1H,s,aromatic protons), 3.63(3H,s,N-CH_3), 2.72(6H,s,>C(CH_3)$_2$).

- $\delta_C(CDCl_3)$; 150.0(s,C-1'), 137.2(s,C-8), 128.0(d), 126.4(d), 126.2(s), 125.5(d), 124.6(s), 121.4(d), 121.2(d), 118.4(d), 109.0(d,C-7), 38.9(s,>C(CH_3)$_2$), 32.5(q,N-CH_3), 30.8(q,>C(CH_3)$_2$).

Route to 5-hydroxy-3-(4-methoxybenzyl)indole (57)

a) 5-Benzyloxy-3-(4-methoxybenzoyl)indole (55)

To a solution of 5-benzyloxyindole (54) (3.35g; 15mmol) in dry diethyl ether (40cm^3) mechanically stirred and under a nitrogen atmosphere was slowly added ethylmagnesium bromide (15mmol), also in dry diethyl ether (10cm^3). The mixture was then stirred for 30 minutes before the dropwise addition of a solution of p-anisoylchloride (2.56g; 15mmol) in dry diethyl ether (20cm^3). After addition was completed, the reaction mixture was refluxed for one hour, cooled, a 10% ammonium chloride solution (5cm^3) carefully added and then stirred for a further ten minutes. The precipitate formed was insoluble in both diethyl ether and ethyl acetate, so it was filtered, washed with diethyl ether (2 x 30cm^3) (to remove any remaining starting material (54)), saturated sodium hydrogen carbonate (2 x 50cm^3) (to remove any 4-methoxybenzoic acid formed) and water

(2 x 50cm^3), refiltered, then dried in an oven at 50°C for three hours to yield a colourless solid (55) (4.3g;80%), recrystallised from 95% ethanol, m.p. 224 - 226°C.

I.R. - ν_{max}(Nujol)cm^{-1}; 3140(br,N-H), 1600(C=O).

M.S. - (low eV,EI)m/z; 357[M$^+$].

N.M.R. - δ_H(d^{6}DMSO)ppm; 11.77(1H,br.s,exchanged by deuteration,N-H), 7.92-7.60 (4H,m,H-2,4,2',6'), 7.60-7.16(6H,m,$C_6\underline{H}_5CH_2O$-,one indole proton), 7.16-6.80(3H,m,H-3',5', and final indole proton), 5.11(2H,s, Ph $C\underline{H}_2O$), 3.81(3H,s,$OC\underline{H}_3$).

- δ_C(d^{6}DMSO)ppm; 118.6(s,$\underline{C}$=O), 161.6(s,C-4'), 154.4(s,C-5), 137.5(s), 135.0(s), 133.0(s), 131.6(s), 130.4(d), 128.3(d), 127.5(d), 114.9(s), 113.5(d), 112.8(d), 104.9(d), 69.7(t,Ph$C\underline{H}_2O$), 55.3 (q,$OC\underline{H}_3$).

b) 5-Benzyloxy-3-(4-methoxybenzyl)indole (56)

The ketone (55) (2.5g; 7mmol) was reduced to the required methylene compound (56) using the diborane reduction previously employed for the reduction of 3-(4-methoxybenzoyl)indole (36). The product of this reaction was purified by column chromatography to yield a light orange gum (56) (1.5g; 62%). The compound was recrystallised using a low temperature diethyl ether/petroleum ether solvent system, but melted when warmed to room temperature.

I.R. - ν_{max}(thin film); 3420(br,N-H).

M.S. - (low eV,EI)m/z; 343(100%,[M$^+$]), 252(81%), 135(11%), 91(9%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 7.60(1H,br.s,exchanged by deuteration,N-H), 7.48-6.52(13H,m,all aromatic protons), 4.93(2H,s,$PhCH_2O$-), 3.90)2H,s,Ind-CH_2), 3.64(3H,s,-OCH_3).

- $\delta_C(CDCl_3)$ppm; 157.9(s,C-4'), 153.0(s,C-5), 137.7(s,C-8), 133.3(s,C-1'), 131.9(s), 129.5)d), 128.3(d), 128.1(s,C-9), 127.7(d), 123.2(d), 115.7(s,C-3), 113.8(d), 112.7(d), 111.8(d), 102.8(d), 71.0(t,$PhCH_2O$-), 55.1(q,-OCH_3), 30.7(t,Ind-CH_2-).

c) 5-Hydroxy-3-(4-methoxybenzyl)indole (57)

A solution of the benzyloxyindole derivative (56) (1.7g; 5mmol) and a catalytic amount of 10% palladium on charcoal in ethyl acetate ($20cm^3$)were stirred under a hydrogen atmosphere at atmospheric pressure. After three hours, TLC analysis showed the reaction to be steadily progressing, so it was left stirring overnight (15 hours). After this time TLC analysis showed the reaction to have gone to completion, so the catalyst was filtered off, the solvent removed and the residue purified by column chromatography to yield some recovered starting material (400mg) and a light orange coloured gum (57) (925mg; 96% corrected yield). All attempts to crystallise the compound failed.

I.R. - ν_{max}(thin film)cm^{-1}); 3410-3200(br,N-H,O-H).

M.S. - (low eV, EI)m/z; 253[M^+].

- (70 eV, EI)m/z; 253(100%,[M^+]), 252(49%), 146(39%).

- Accurate mass: $C_{16}H_{15}NO_2$; Requires 253.1101
  Found 253.1092.

N.M.R. - $\delta_H(CDCl_3)$ppm; 7.81(1H,br.s,exchanged by deuteration,N-H), 7.10-6.56(8H,m,all aromatic protons), 5.90(1H,s,exchanged by deuteration,O-H), 3.85(2H,s,CH_2), 3.65(3H,s,OCH_3).

- $\delta_C(CDCl_3)$ppm; 157.6(s,C-4'), 149.3(s,C-5), 133.5(s), 131.8(s), 129.5(d,C-2',6'), 128.1(s), 123.5(d,C-2), 115.3(s), 113.8,111.8 (2d,C-4,6+C-3',5'), 103.7(d,C-7).

Attempted chemical oxidation of 3-benzylindole (21)

a) Standard procedure[32]

The substrate (21) (1.2g; 6mmol), iron II sulphate (360mg; 1.3mmol), EDTA(disodium salt) (2.4g; 6.5mmol) and ascorbic acid (2.5g, 14mmol) were shaken at 37°C in a 0.1m pH 6.7 phosphate buffer (250cm^3) under an oxygen atmosphere at atmospheric pressure for two hours. During this time the indole remained as an insoluble suspension which was filtered at the end of the reaction time to yield complete recovery of the starting material (21).

b) Acetone

The reaction was repeated with less phosphate buffer (100cm^3) and sufficient acetone to dissolve the substrate (100cm^3). The reaction time was also extended to 15 hours. After this time the acetone was removed under reduced pressure and the resultant precipitate filtered and dried, to again yield complete recovery of the starting material.

c) Dichloromethane/phase transfer catalyst

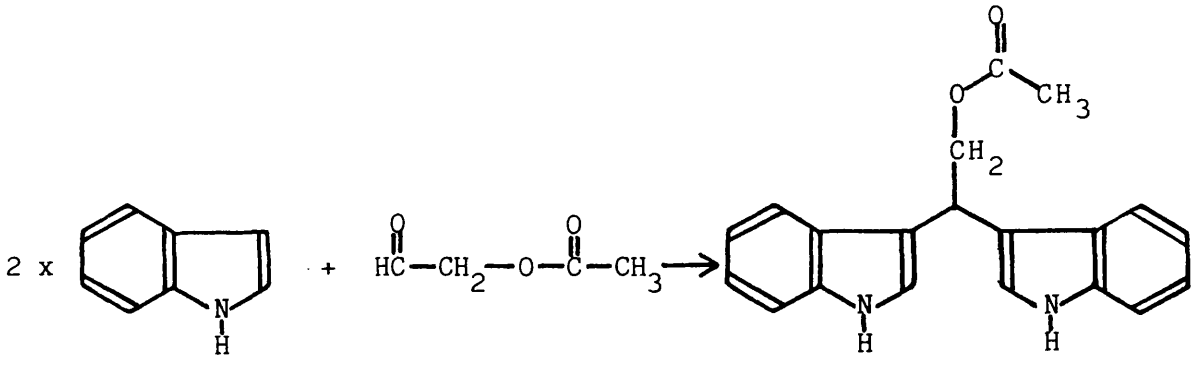
The reaction was then repeated using the original conditions with the addition of dichloromethane (100cm^3) and a phase transfer catalyst, namely cetyltrimethylammonium bromide (0.7g). Shaking was again carried out for 15 hours, after which time the reaction was allowed to settle and the two phases separated. The aqueous phase was extracted with dichloromethane

($2 \times 50cm^3$), and then discarded. The organic fractions were combined, washed with water ($2 \times 50cm^3$), dried ($MgSO_4$) and evaporated to yield a quantitative recovery of the starting material (21).

APPENDIX 1

Synthesis of Streptindole (58)

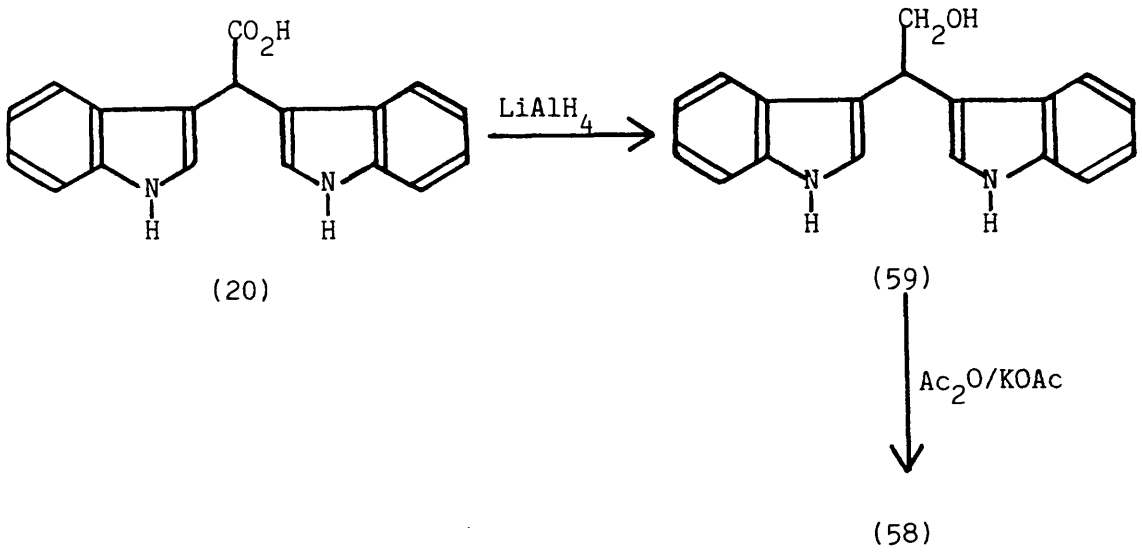
Recently Osawa and Namiki[38] have reported the isolation of streptindole (58), a genotoxic metabolite of human intestinal bacteria. As proof of the structure, Osawa and Namiki synthesised streptindole (58) by condensing indole with acetoxyacetaldehyde (Scheme 15). The yield was only 2%, and thus further testing work on this structure was hampered by lack of substrate.



(58)

SCHEME 15

This low productivity is due to the limited reactivity of the aldehyde used. We had, however, already prepared 3,3'-diindolylacetic acid (20) in excellent yield by a similar condensation reaction and therefore we were in a good position to prepare the streptindole by a simple elaboration of this compound (Scheme 16).



SCHEME 16

In practice, however, direct reduction of the acid (20) proved troublesome and inefficient and so it was first converted to the ester (60) using methanol and thionyl chloride (63% yield). The ester was then cleanly reduced to the alcohol (59) (81%) and O-acetylated (87%) with acetic anhydride to give streptindole (58) in an overall yield of 45%. Spectral data for this compound corresponded almost exactly to those reported[38] for the natural product.

A sample of streptindole (58) was then sent for anti-oxidant appraisal and was shown to be reasonably active, particularly in the iron/ascorbic acid oxidation system (50%I, 5.5μm) (see appendix 2).

Experimental to Appendix 1

Methyl 3,3'-diindolylacetate (60)

To a solution of 3,3'-diindolylacetic acid (20) (5g, 17mmol), stirred in dry methanol ($75cm^3$) at -20°C, was added dropwise thionyl chloride (6g, 51mmol). After the addition was completed, the cooling bath was removed and the reaction mixture allowed to warm to room temperature overnight (15 hours). The solvent and any excess thionyl chloride were then removed and the residue purified by column chromatography to yield a yellow gum (60) (3.3g, 63%).

I.R. - ν_{max}(thin film)cm^{-1}; 3400(N-H), 1720(C=O).

M.S. - (low eV, EI)m/z; 304(98%,[M^+]), 245(59%), 217(28%), 117(100%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 8.00(2H,br.s,exchanged by deuteration,N-H), 7.72-7.47(2H,m), 7.23-6.83(8H,m,rest of indole protons), 5.40(1H,s,CH), 3.70(3H,s,OCH_3).

2,2-(3,3'-Diindolyl)ethanol (59)

To a suspension of lithium aluminium hydride (3g, 8mmol) in dry diethyl ether ($50cm^3$) stirred in an ice bath and under a nitrogen atmosphere was added a solution of the ester (60) (3g, 10mmol) in dry diethyl ether ($50cm^3$). After the addition, the ice bath was removed and the mixture stirred for a further 20 minutes before a saturated solution of sodium potassium tartrate ($10cm^3$) was carefully added. The organic layer was then separated and the residue washed with chloroform (2 x $50cm^3$). The organic fractions were then combined, washed with water ($50cm^3$), dried ($MgSO_4$) and evaporated to yield a colourless gum, which was purified

by column chromatography to yield the required alcohol (59) (2.2g, 81%) as a colourless solid, crystallised from toluene/petroleum ether, m.p. 50°C.

I.R. - ν_{max}(Nujol)cm^{-1}; 3400(sh,N-H), 3400-3100(br,OH).

M.S. - (low eV, EI)m/z; 276(33%,[M^+]), 245(100%), 244(38%), 194(21%).

N.M.R. - δ_H($CDCl_3$)ppm; 7.68(2H,s,N-H), 7.48-7.32(2H,m), 7.08-6.84(6H,m), 6.48(2H,s), 4.54(1H,t(J=6Hz),CH), 4.02(2H,d(J=6Hz),CH_2OH).

Streptindole (58)

A mixture of the alcohol (59) (300mg, 3mmol), dried potassium acetate (1g) and freshly distilled acetic anhydride ($5cm^3$) were stirred at room temperature for 17 hours. The majority of the acetic anhydride was removed under reduced pressure and the residue stirred with ethyl acetate ($20cm^3$) and ethanol ($3cm^3$) overnight. The solution was then washed with water (2 x $10cm^3$), 5% sodium hydrogen carbonate (3 x $10cm^3$), dried ($MgSO_4$) and evaporated to yield streptindole (58) (800mg, 87%) as a low melting solid.

U.V. - λ_{max}nm; 290, 283, 273.
lit[38]; 290, 283, 273.

I.R. - ν_{max}($CHCl_3$)cm^{-1}; 3500(N-H), 1730(C=O), 1620
lit[38]; 3400(N-H), 1730(C=O), 1620).

M.S. - Accurate mass: $C_{20}H_{18}N_2O_2$; requires 318.1368
found 318.1374.

N.M.R. - δ_H($(CD_3)_2$,CO)ppm; 9.94-).75(2H,br.s,N-H), 7.55-6.70(10H,m, indole protons), 4.80-4.50(3H,m,CHCH$_2$O), 1.93(3H,s,CH_3CO).

APPENDIX 2

Biological Tests on Indole Inhibitors

It is known that many toxicological and carcinogenic responses of organisms to chemicals are often mediated by free radical species. One easily monitored parameter resulting from the generation of free radicals is lipid peroxidation. In experiments using indole-3-carbinol, Shertzers et al[9] have shown that there is a high degree of correlation (r^2=0.82) between the decrease in the covalent binding of NDMA and the decrease in lipid oxidation.

Our compounds were tested for their ability to prevent lipid peroxidation in the belief that, as in the case of indole-3-carbinol (8), it would be a true reflection of their cancer inhibiting properties.

Two oxidation systems were used:-

1) a hydrophobic system containing phospholipid dissolved in chlorobenzene, with oxidation initiated by the thermal and photo decomposition of azobisisobutyronitrile (AIBN), a well known radical initiator.
2) an aqueous system containing sonicated phospholipid in phosphate buffer (pH 7.4), with oxidation initiated by ferrous/ascorbate.

Increasing concentrations of each of our compounds were added to both lipid/oxidation systems. After a set period of time, the reactions were stopped by the addition of BHT (a powerful anti-oxidant) and the degree of lipid oxidation recorded and plotted against a control of the pure lipid/oxidation system. The results are given in graphical form and have been graded on the concentration required to inhibit 50% lipid oxidation for both oxidation systems.

TABLE 6

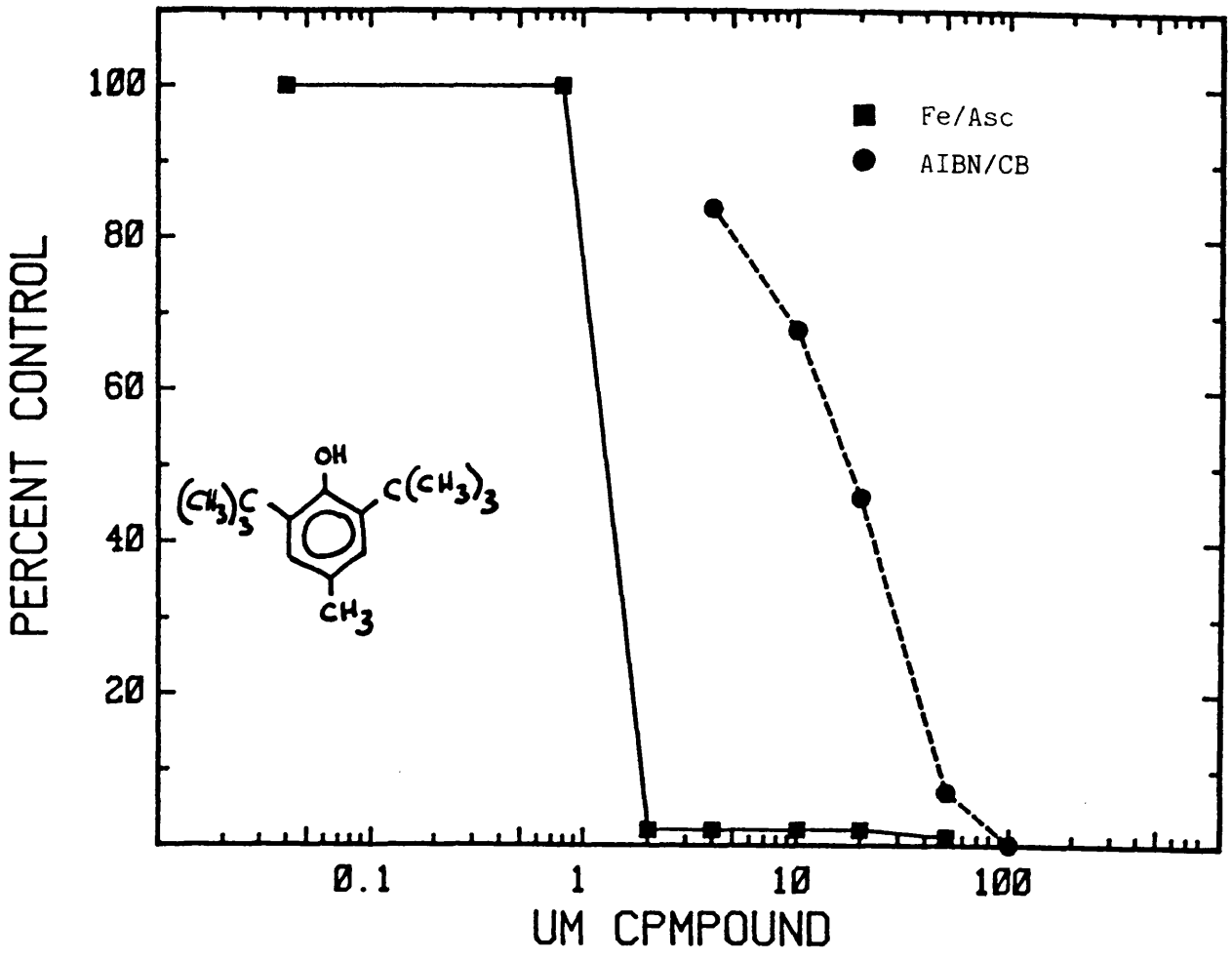
Activity of compounds ranked by the iron/ascorbic acid oxidation system

Compound	Conc. 50% I (μm)
5,10-Dihydroindeno[1,2-b]indole (52)	<4
Butylated hydroxytoluene (15)	1.2
3-(4-Dimethylaminobenzyl)indole (35)	1.5
Streptindole (58)	5.5
∝-Tocopherol	10
3-(2,4,6-Trimethylbenzyl)indole (24)	11
3-(4-Hydroxybenzyl)indole (34)	12
3-[2-(3,4-Dimethoxyphenyl)ethyl]indole (38)	12
3-Benzyl-1-methylindole (49)	13
3,3'-Diindolylmethane (7)	15
3-(2-Methyl-2-phenylethyl)indole (39)	18
3-(4-Methoxybenzyl)indole (22)	24
3-(4-Methoxybenzyl)-1-methylindole (50)	31
3-Benzylindole (21)	36
Indole-3-carbinol (8)	300
3-(2-Pyridinylmethyl)indole (26)	325
3-(3-Pyridinylmethyl)indole (33)	500
Indole	800
3-(4-Methoxybenzoyl)indole (36)	250++
Indole-3-acetonitrile (6)	1250+
1-Methyl-3-(2-methyl-2-phenylethyl)indole (51)	1250+
Skatole (25)	-

TABLE 7

Activity of compounds ranked by chlorobenzene/AIBN oxidation system

Compound	Conc. 50% I (μm)
3-(4-Hydroxybenzyl)indole (34)	13
5,10-Dihydroindeno[1,2-b]indole (52)	14
3-(4-N,N-Dimethylaminobenzyl)indole (35)	16
Butylated hydroxytoluene (15)	18
α-Tocopherol	40
3,3'-Diindolylmethane (7)	64
3-(2-Methyl-2-phenylethyl)indole (39)	80
3-(2,4,6-Trimethylbenzyl)indole (24)	100
3-[2-(3,4-Dimethoxyphenyl)ethyl]indole (38)	115
Indole-3-carbinol (8)	120
3-Benzylindole (21)	185
3-(2-Pyridinylmethyl)indole (26)	200
Streptindole (58)	250
3-(4-Methoxybenzyl)indole (22)	300
3-(4-Methoxybenzyl)-1-methylindole (50)	400
3-Benzyl-1-methylindole (49)	500
3-(3-Pyridinylmethyl)indole (33)	500
Indole	1250
Indole-3-acetonitrile	1250
3-(4-Methoxybenzoyl)indole (36)	250++
1-Methyl-3-(2-methyl-2-phenylethyl)indole (51)	1250+
Skatole (25)	-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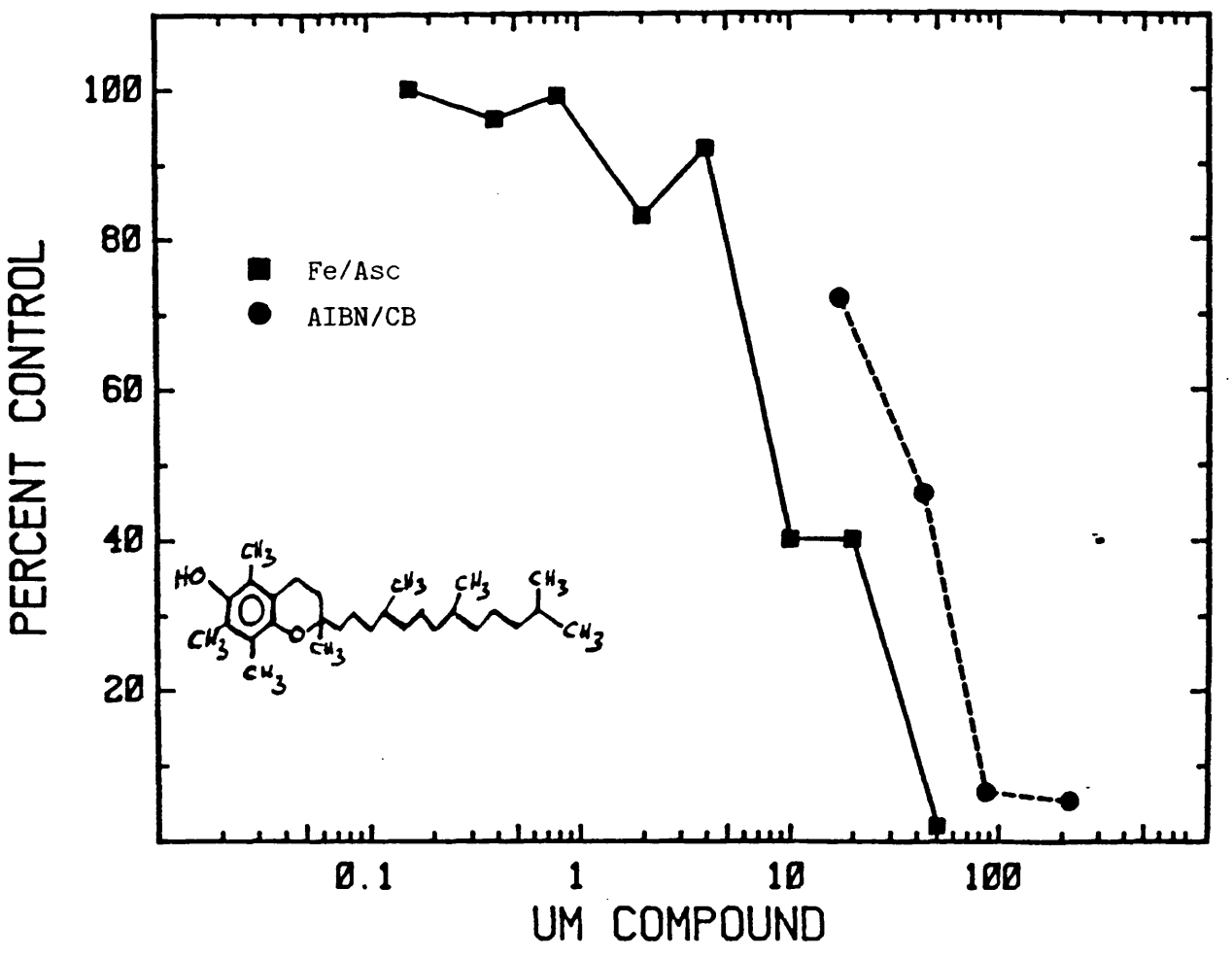
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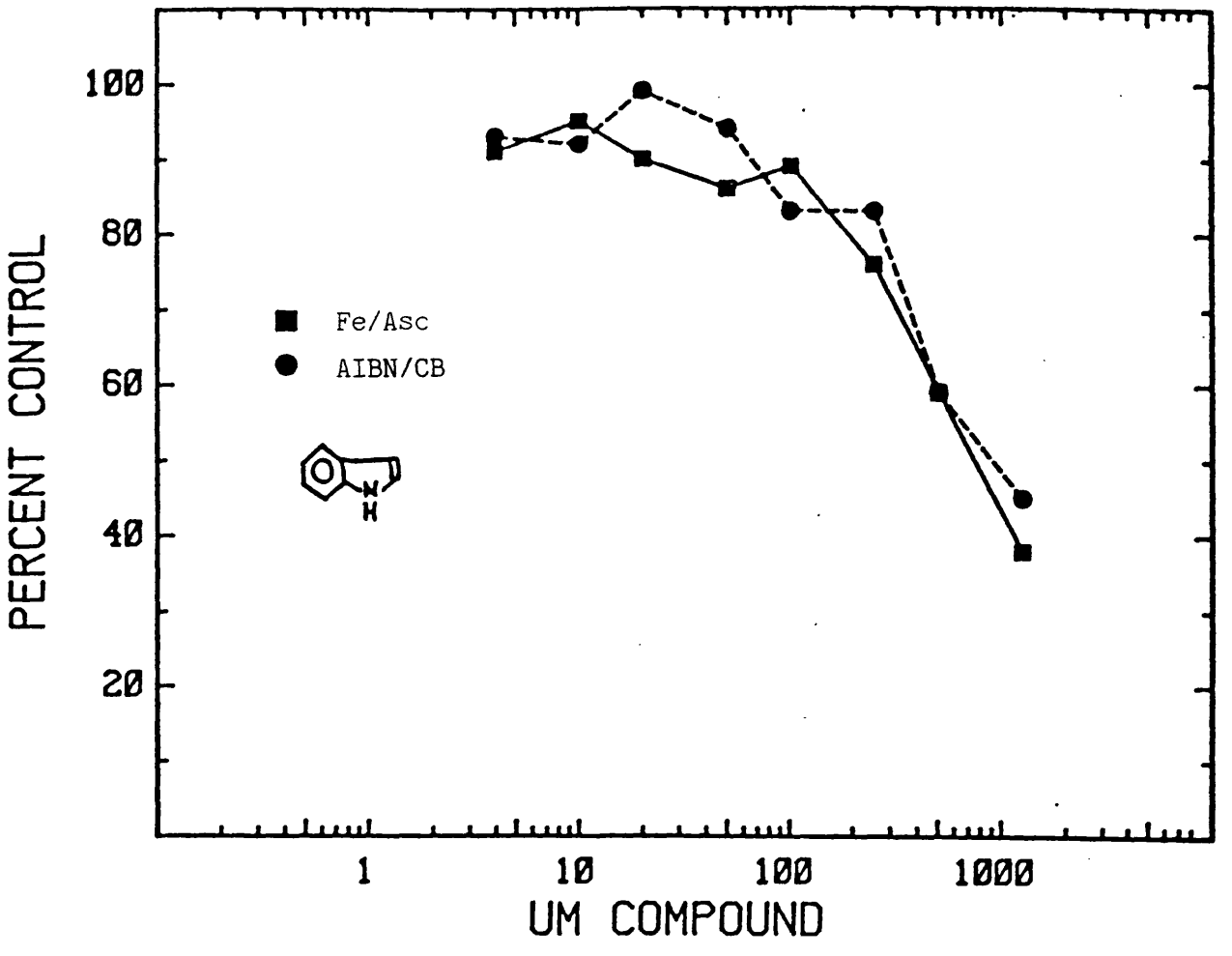
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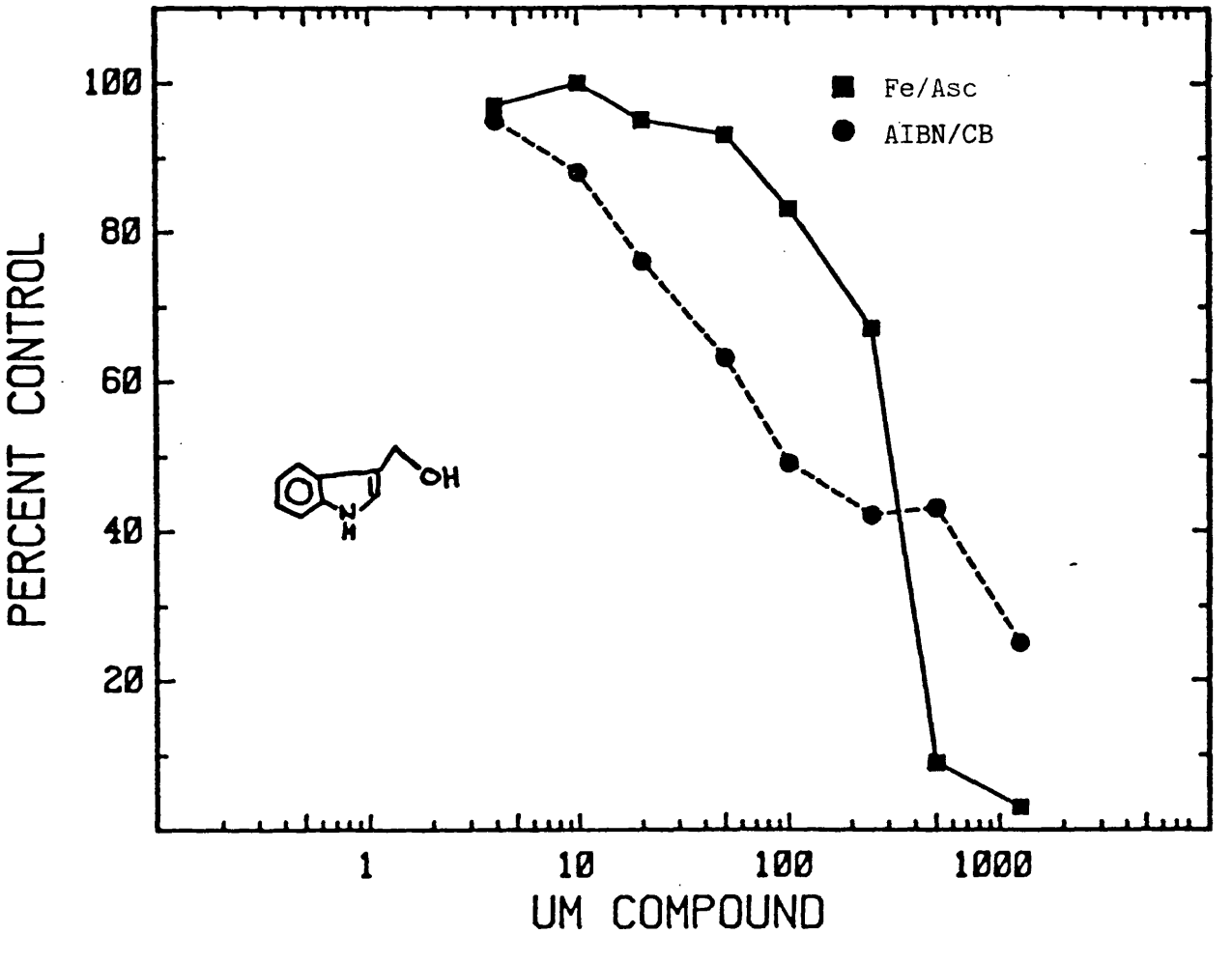
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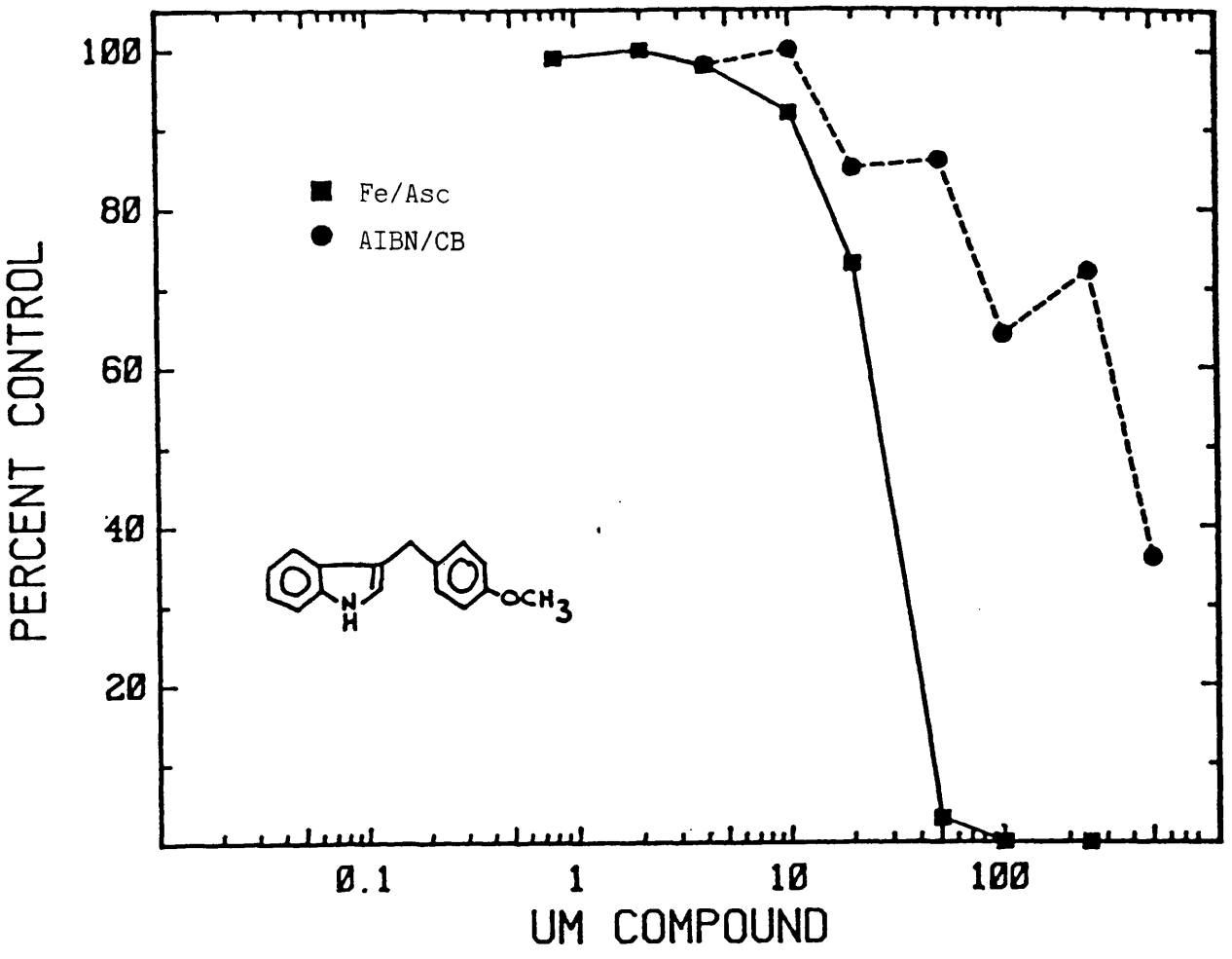
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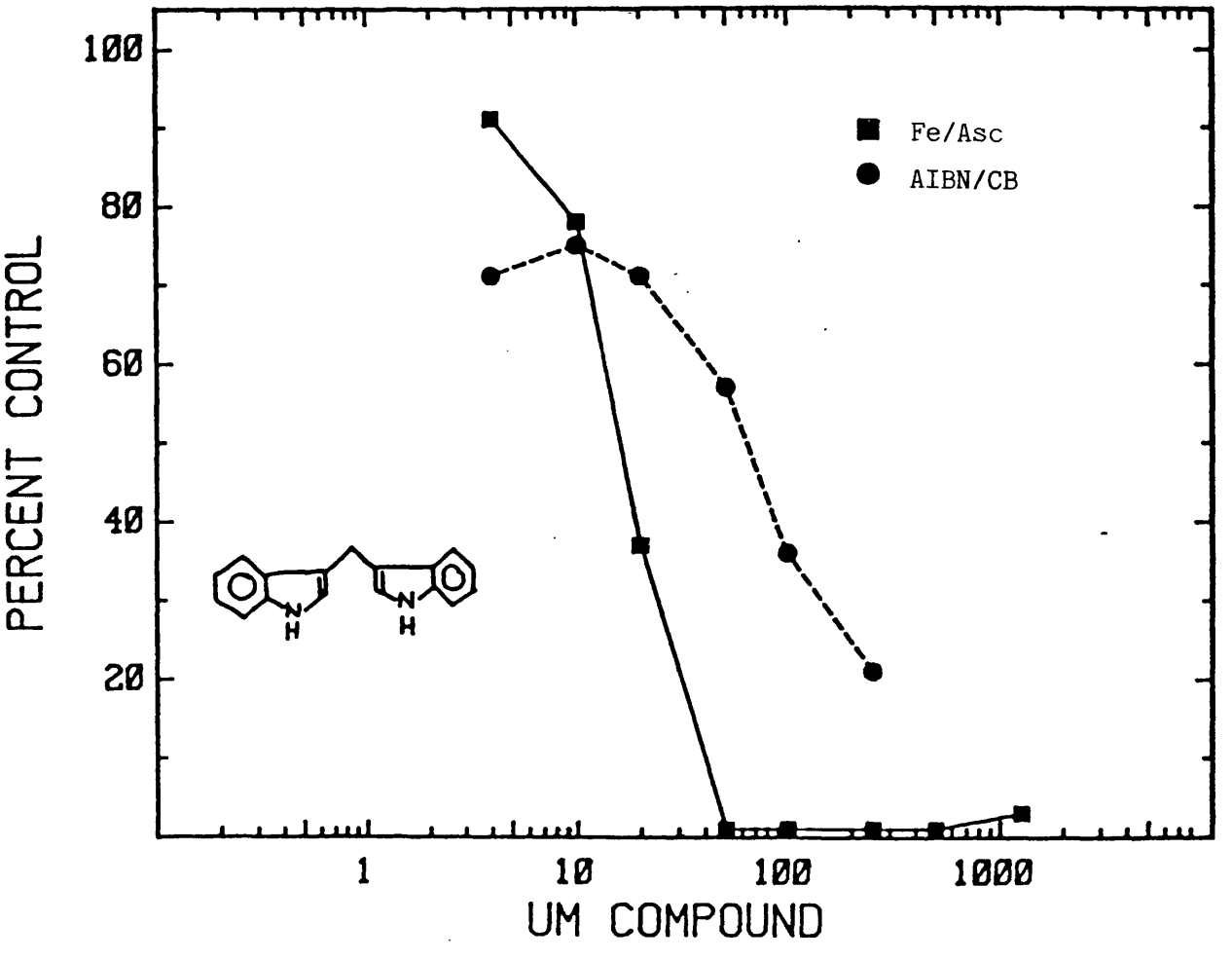
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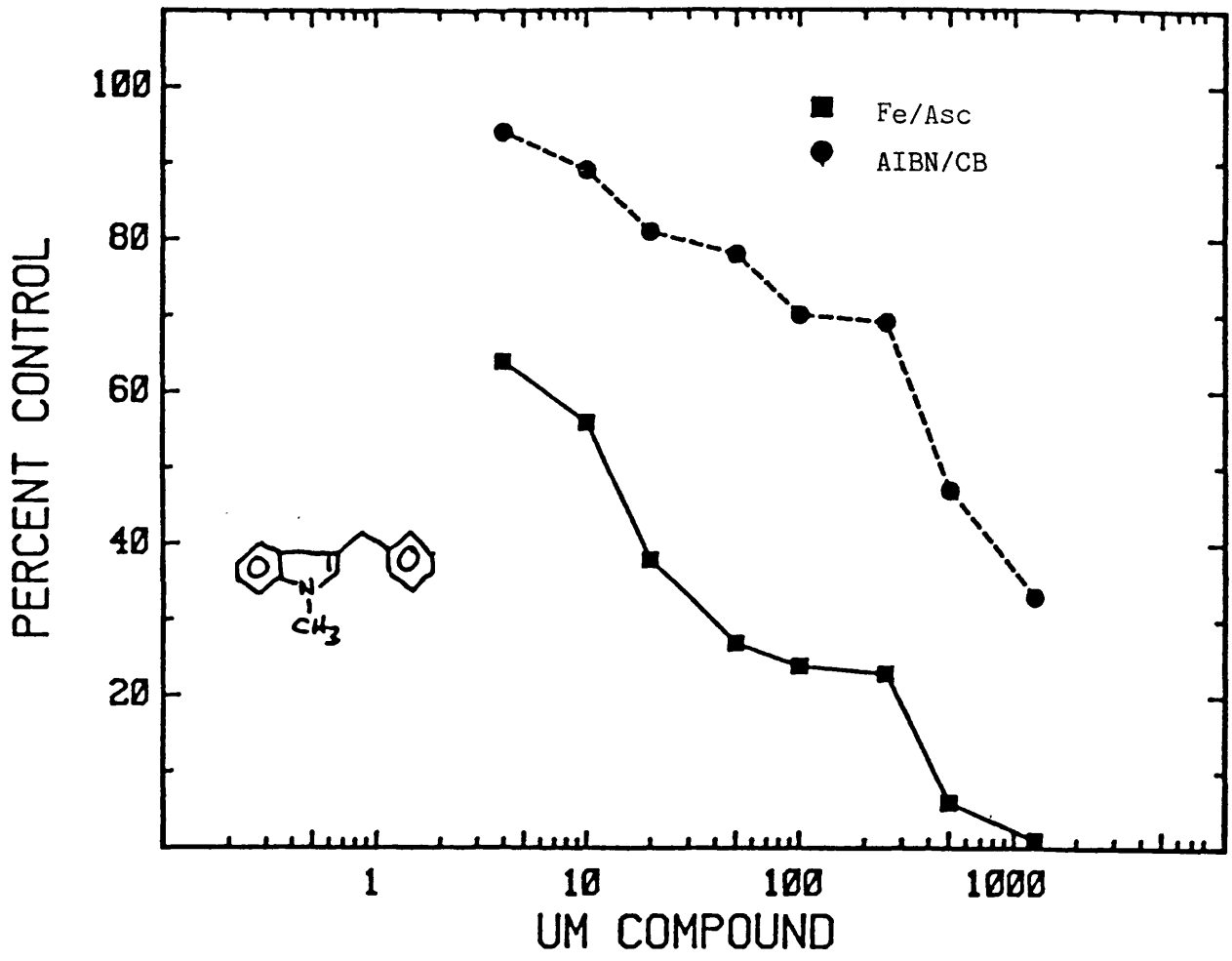
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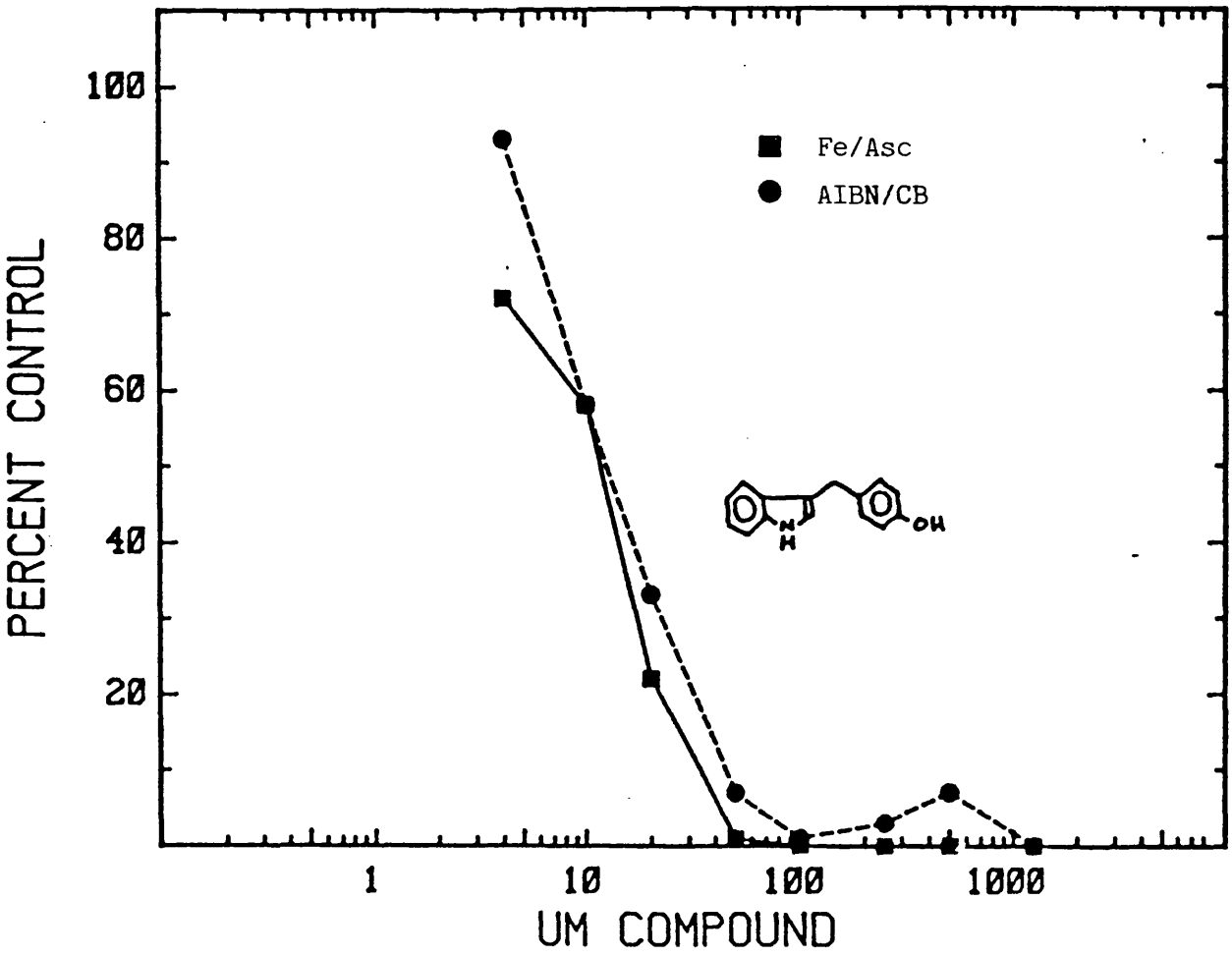
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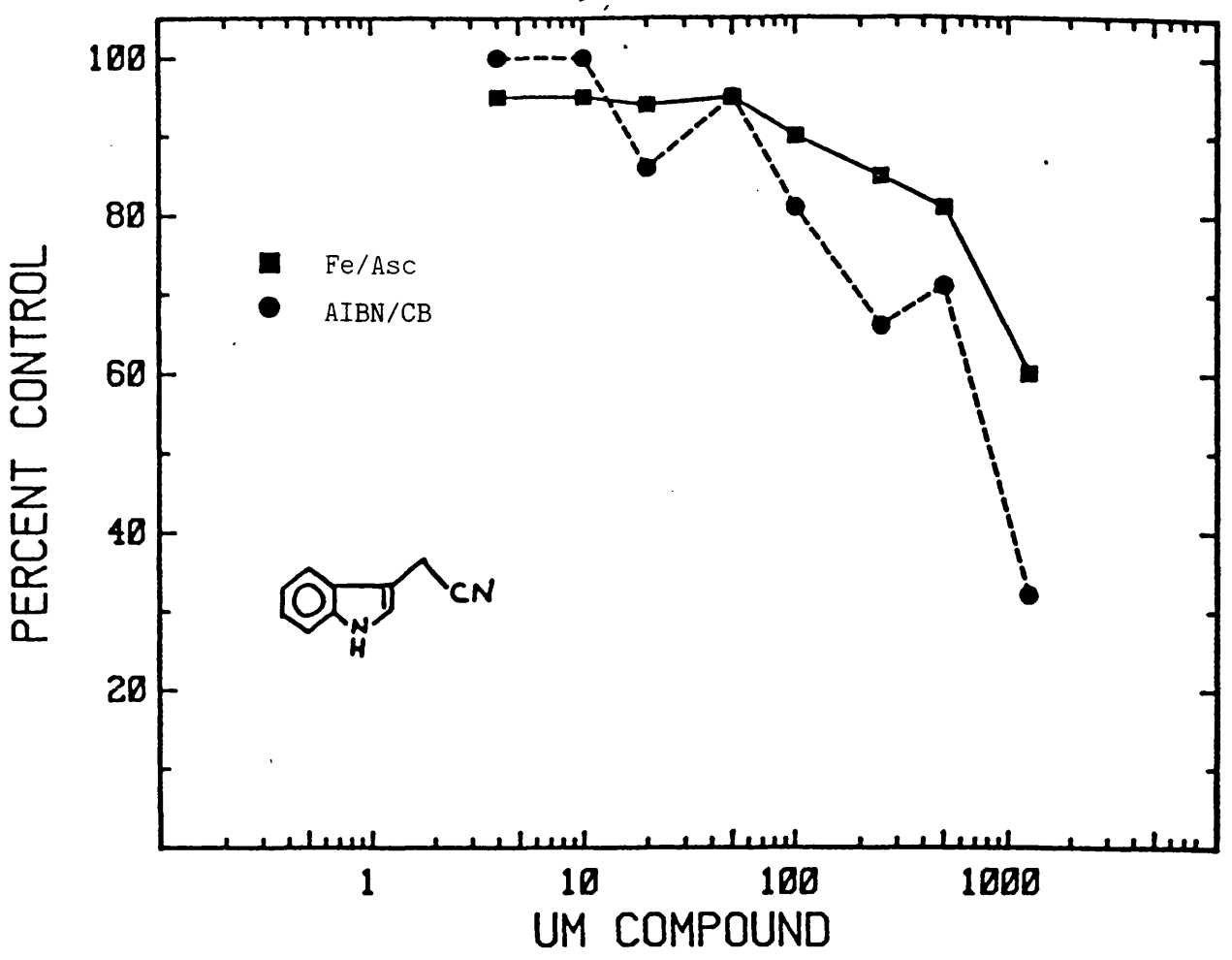
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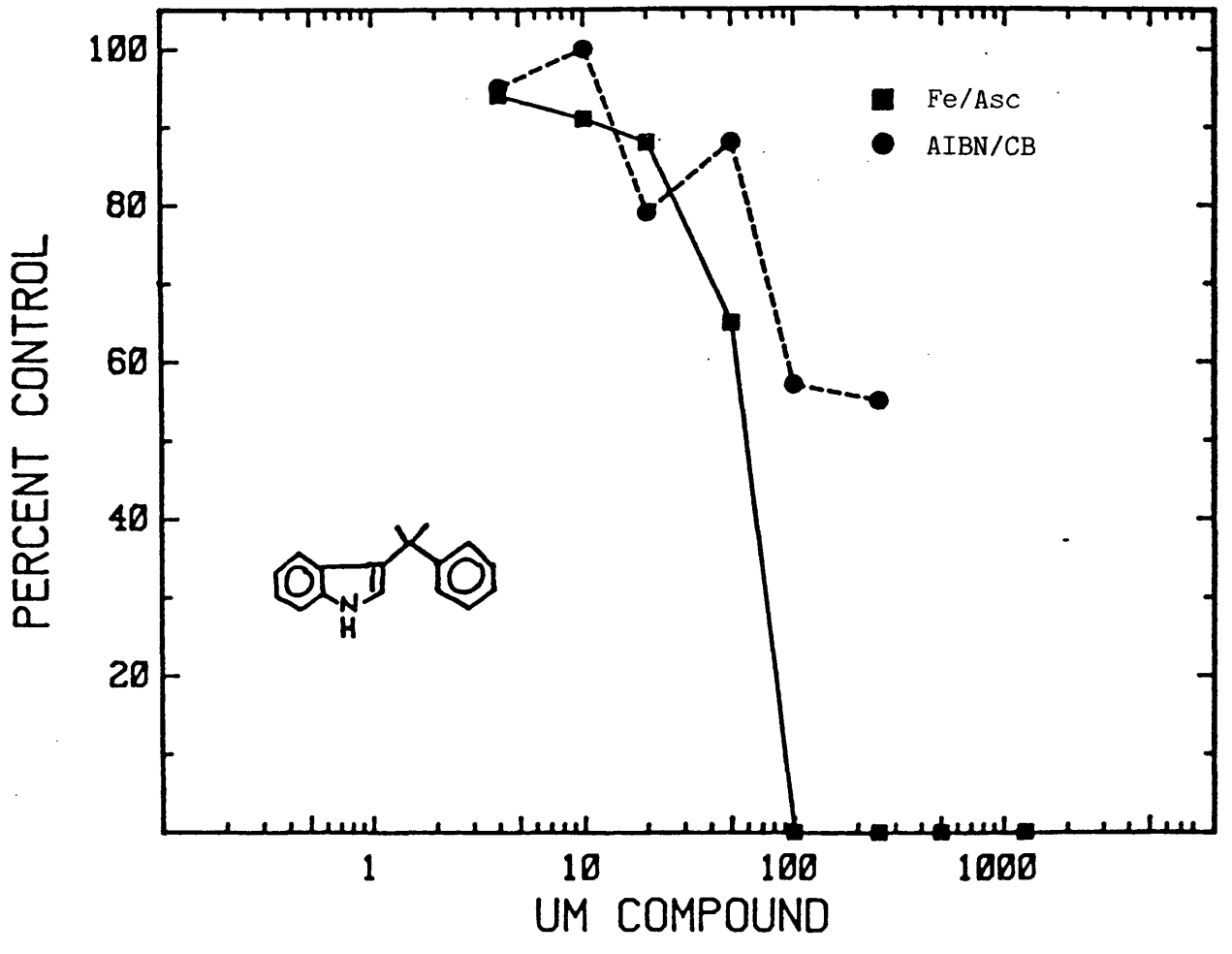
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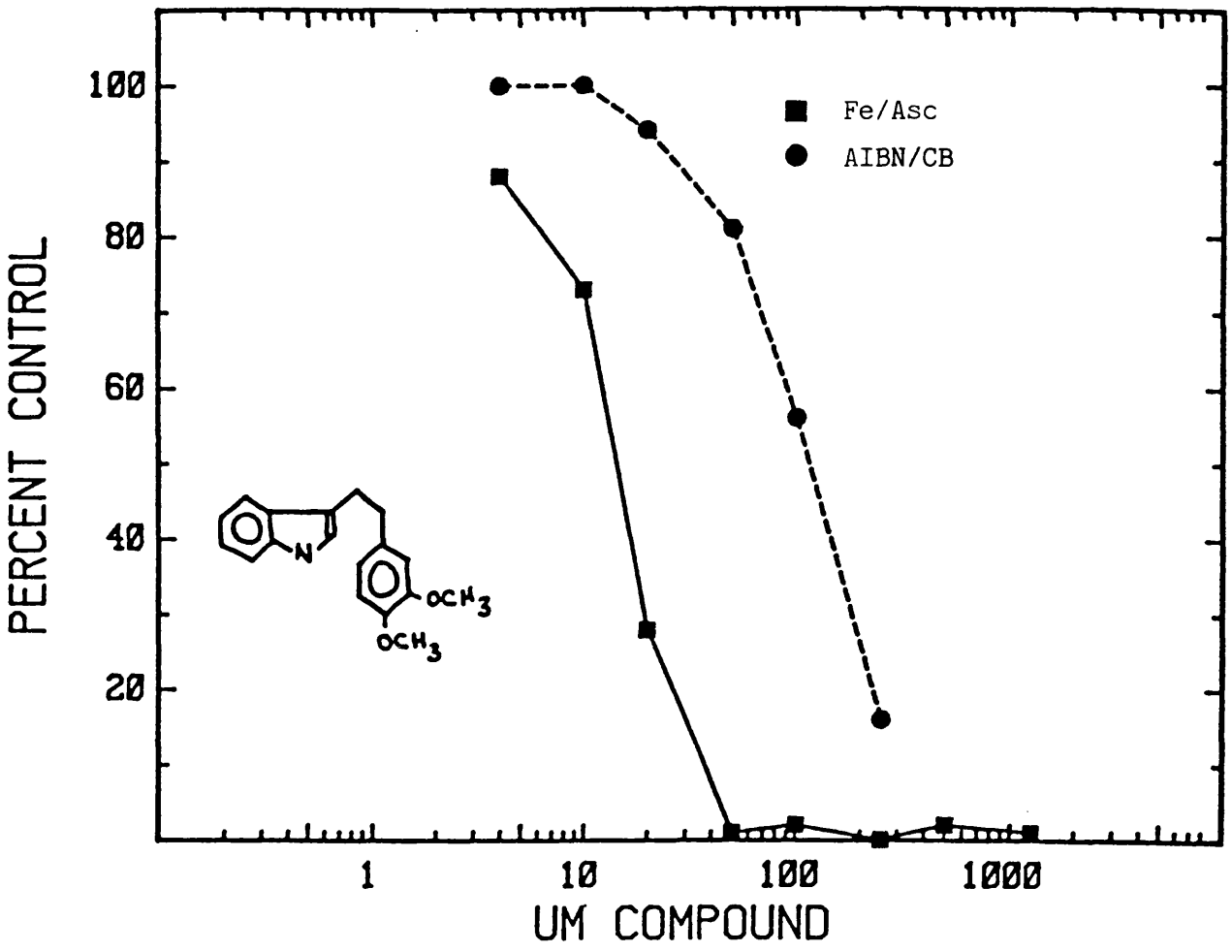
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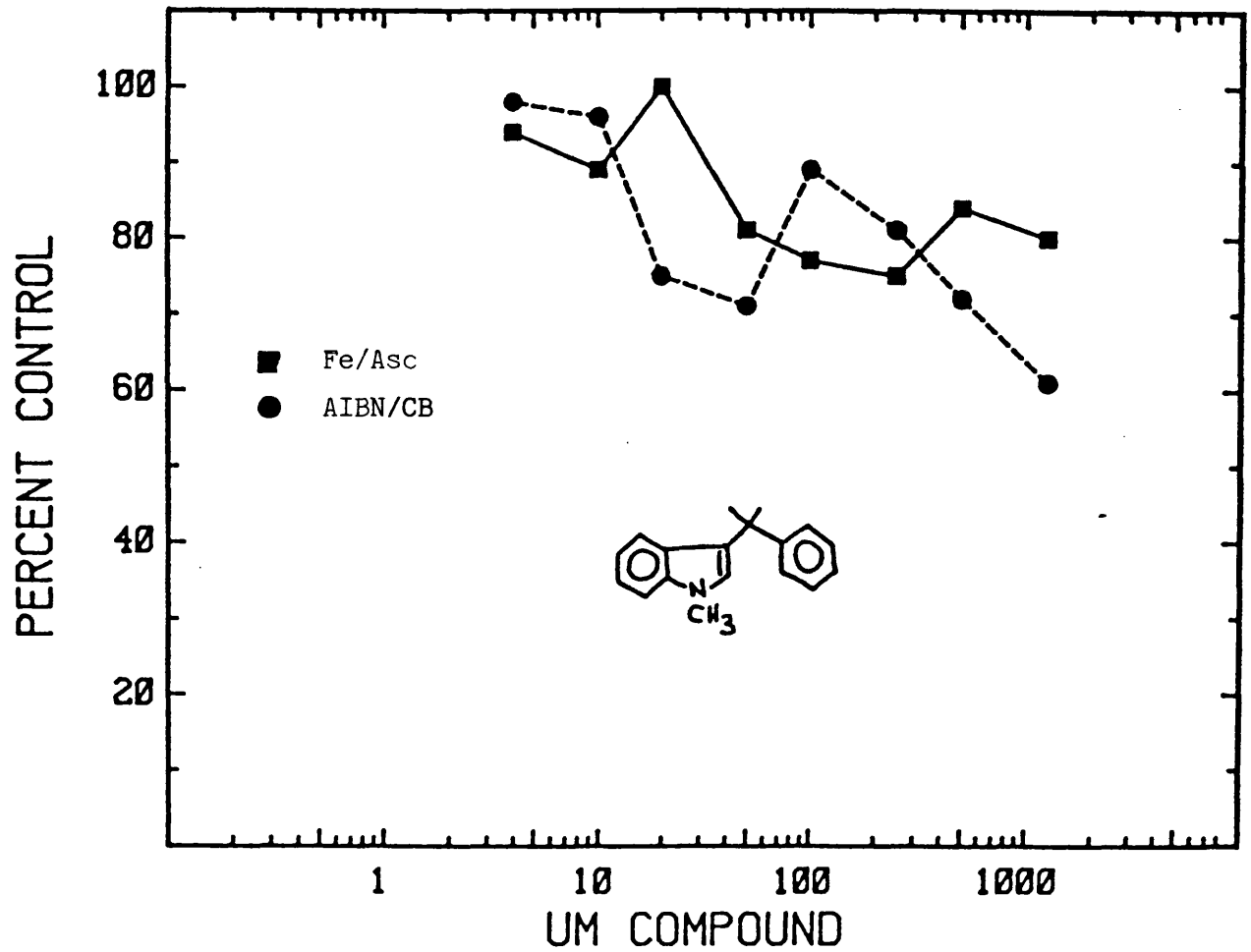
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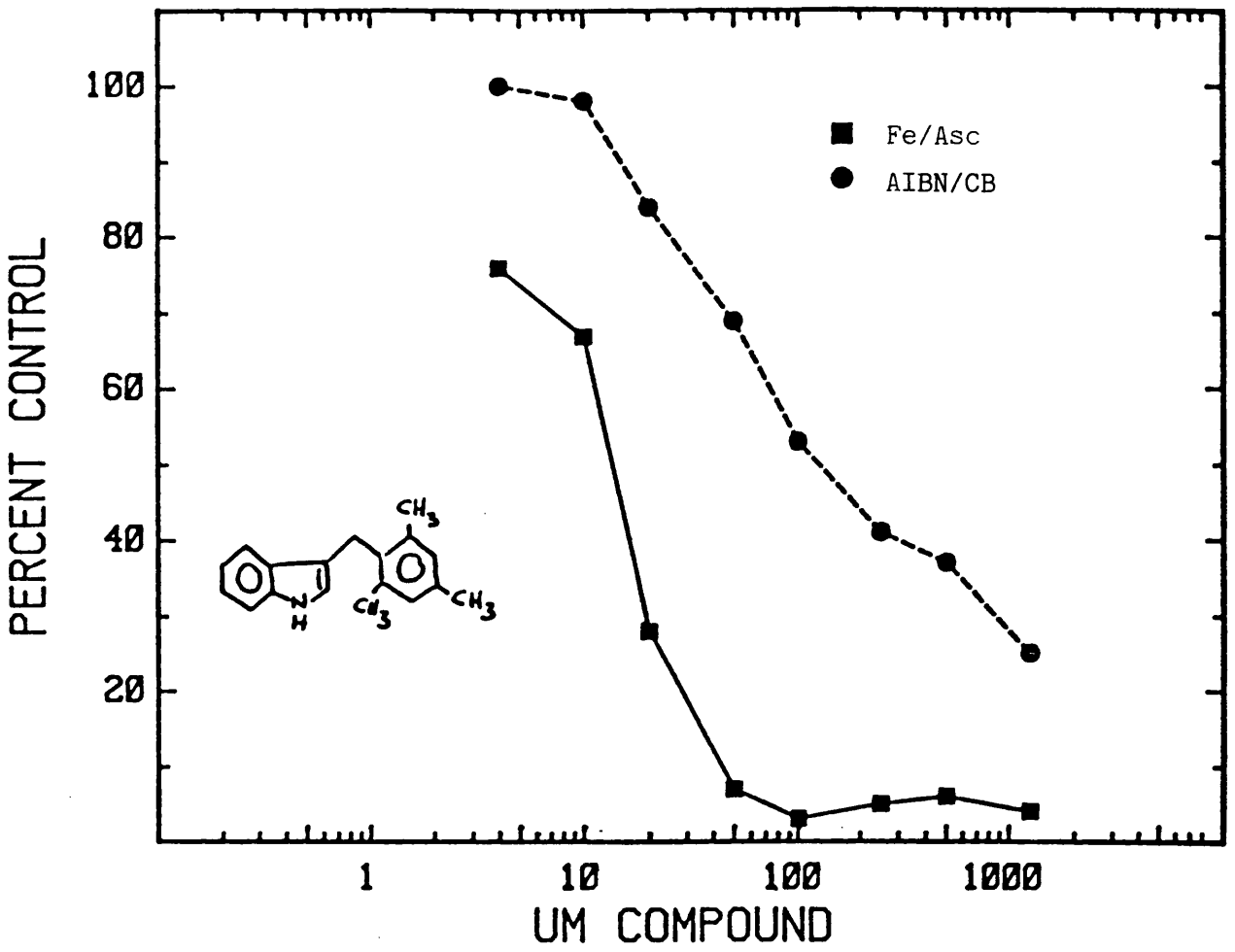
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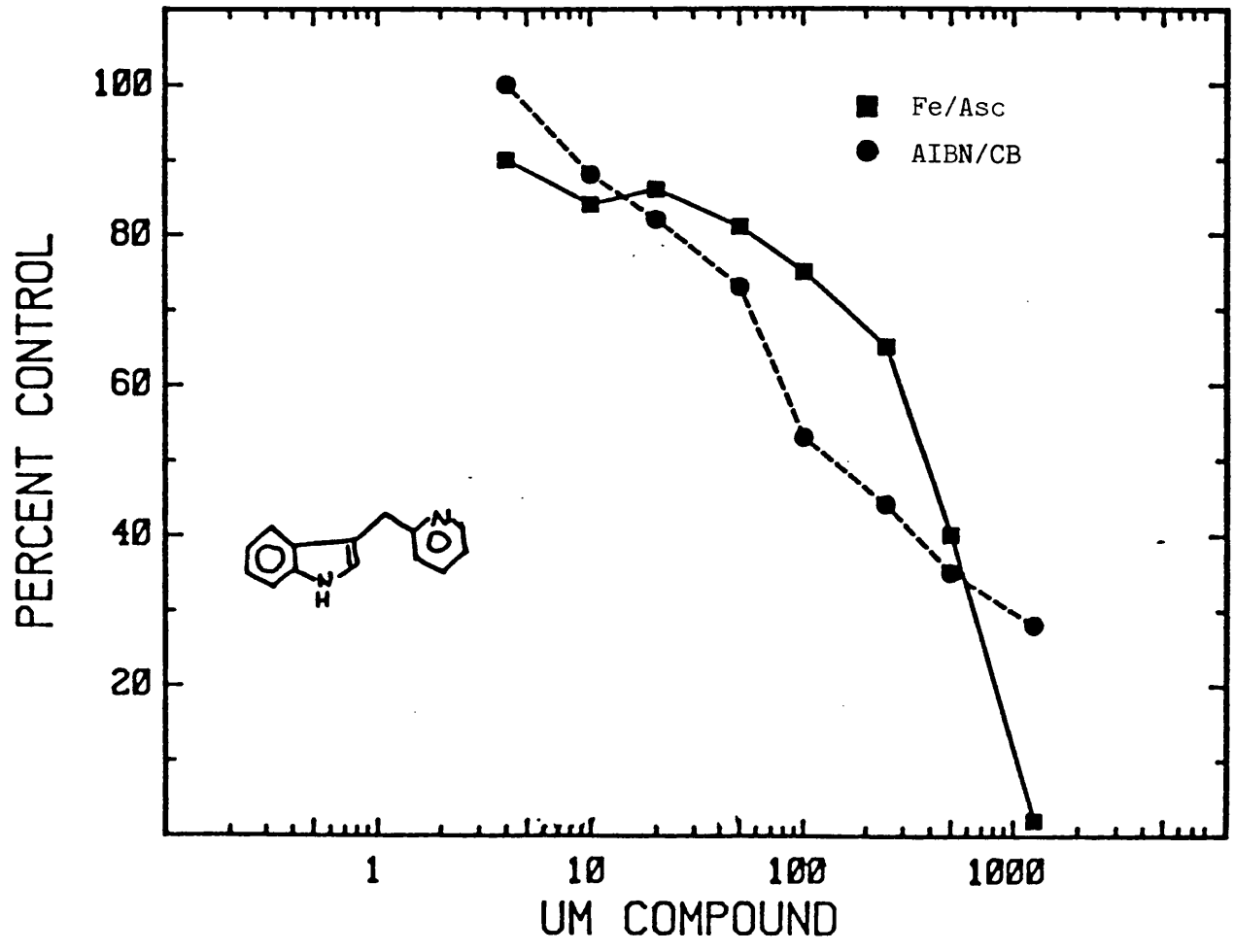
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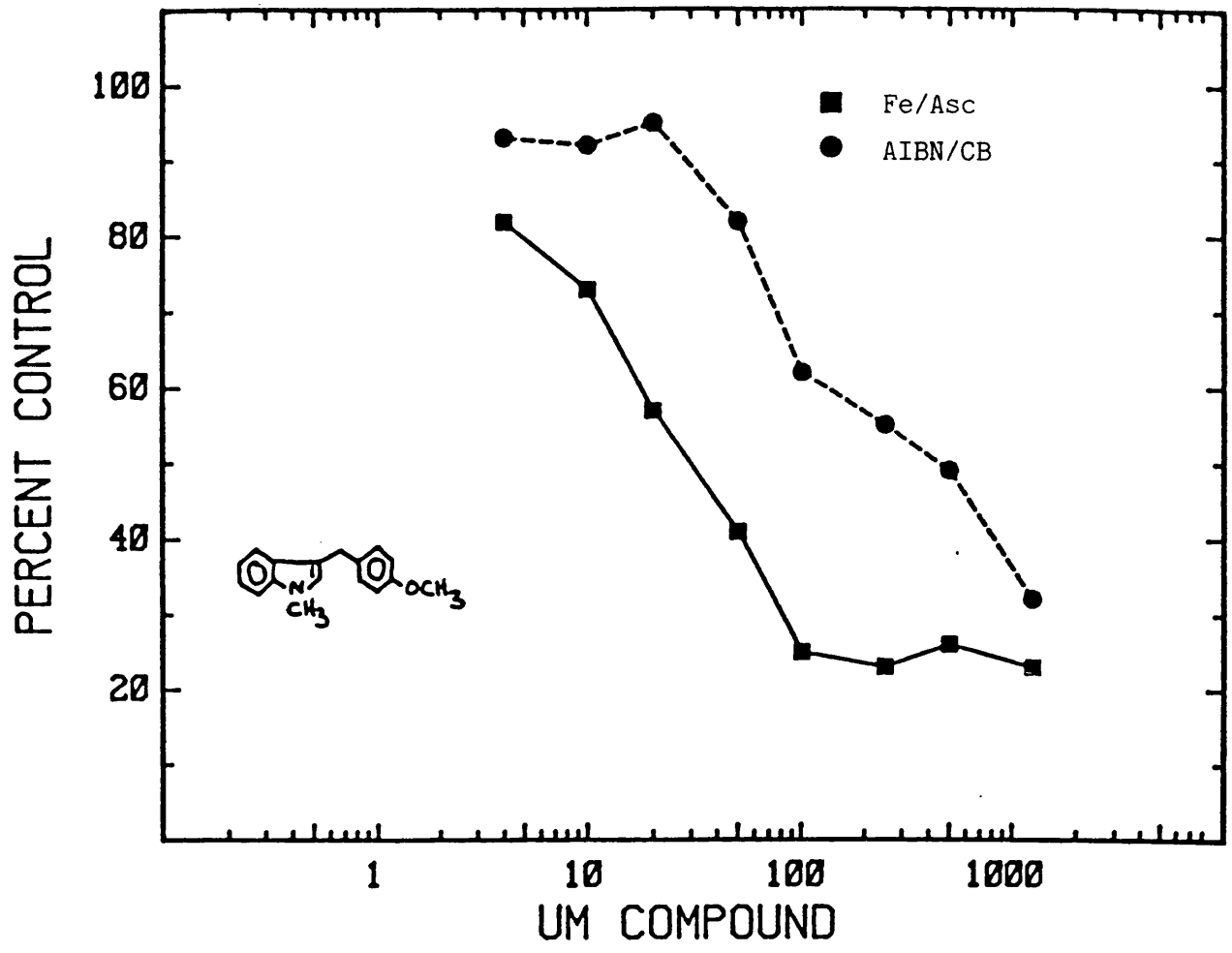
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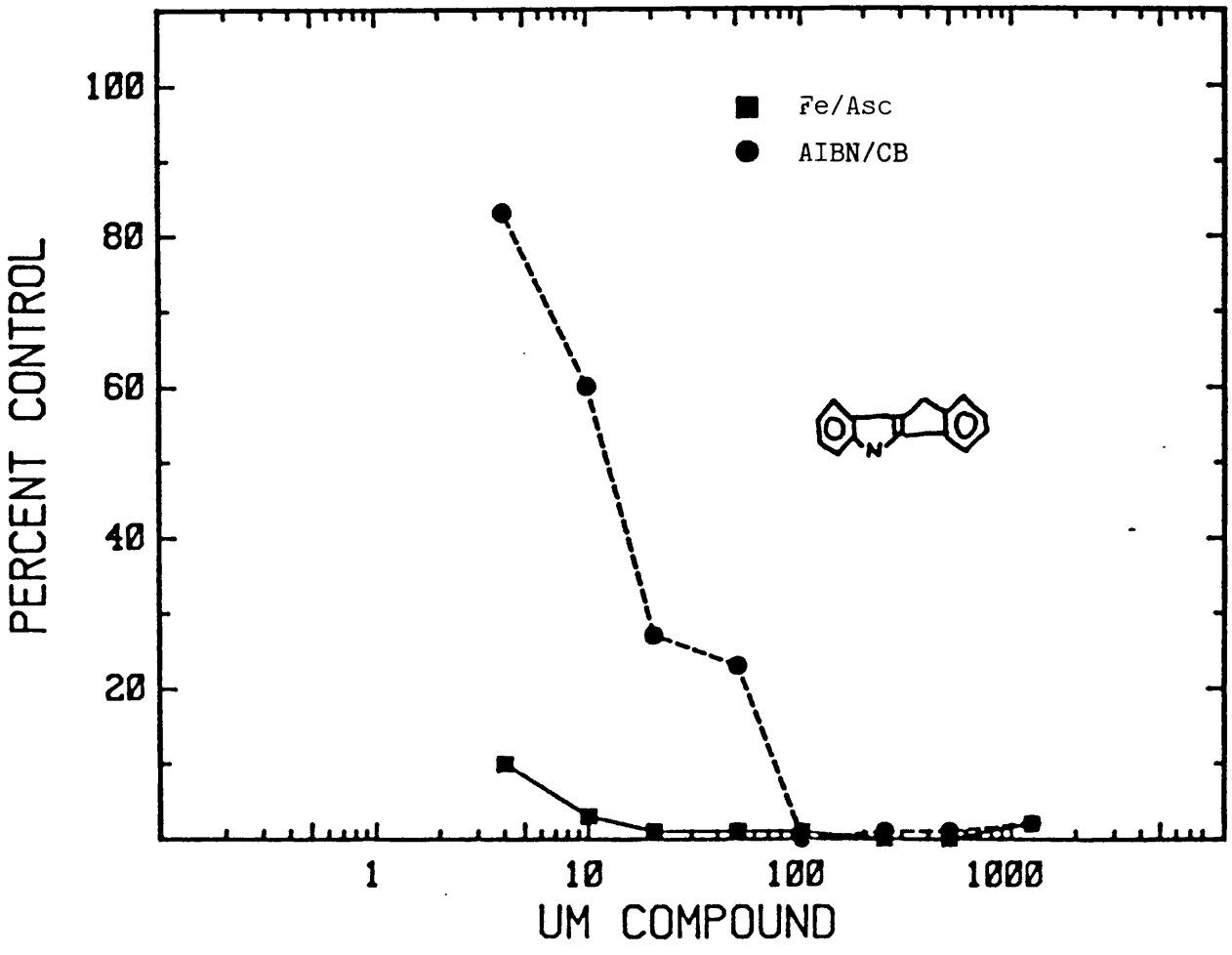
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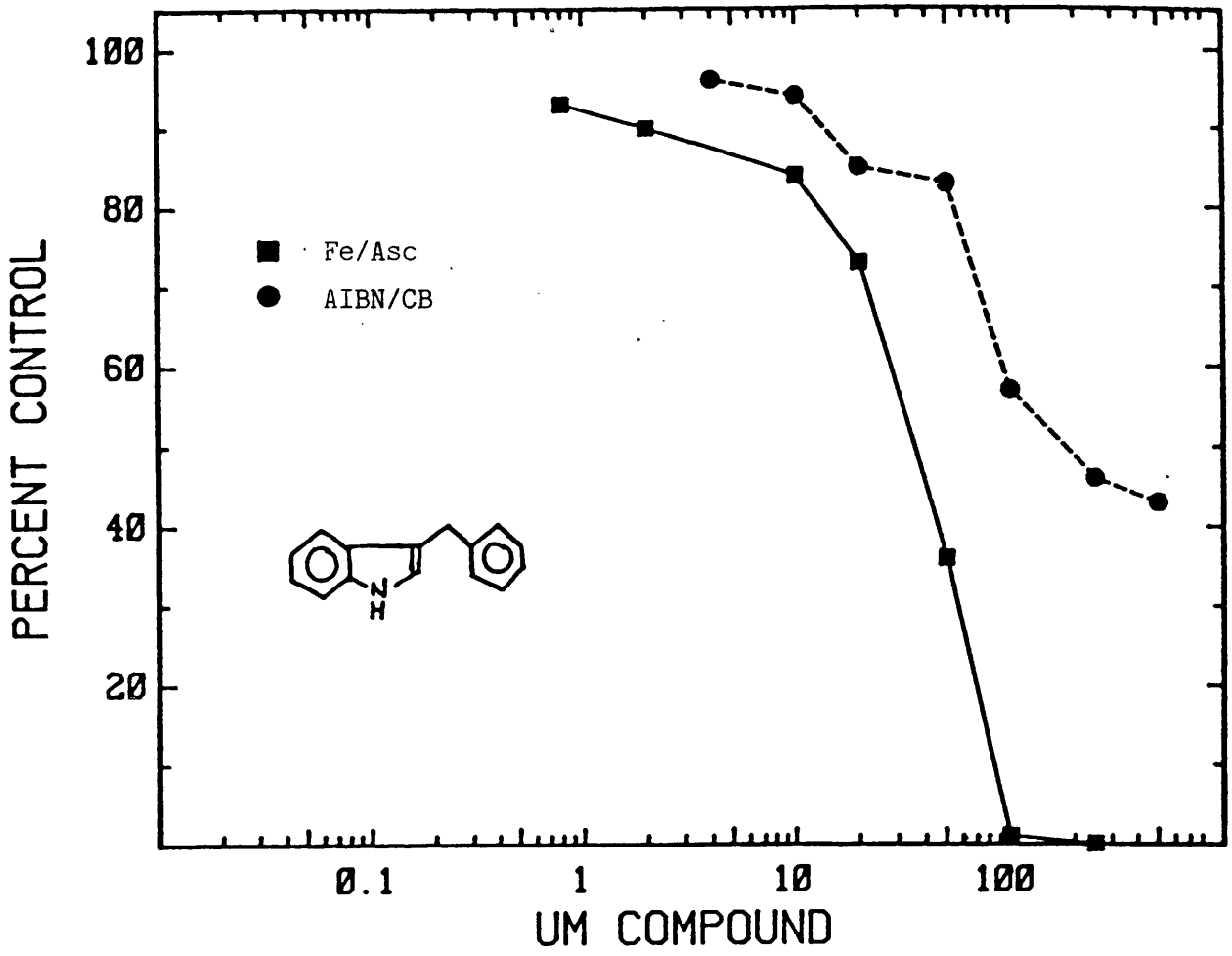
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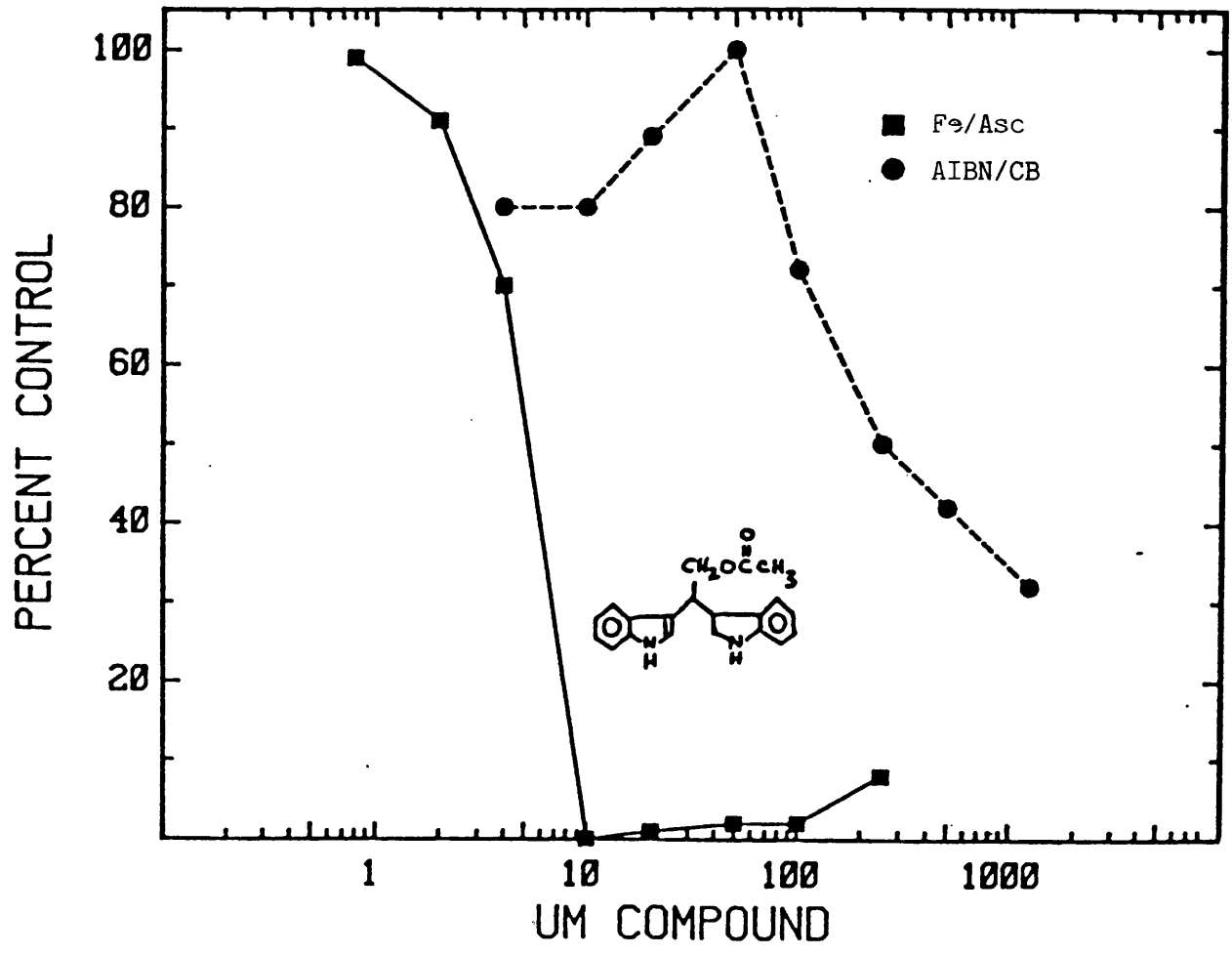
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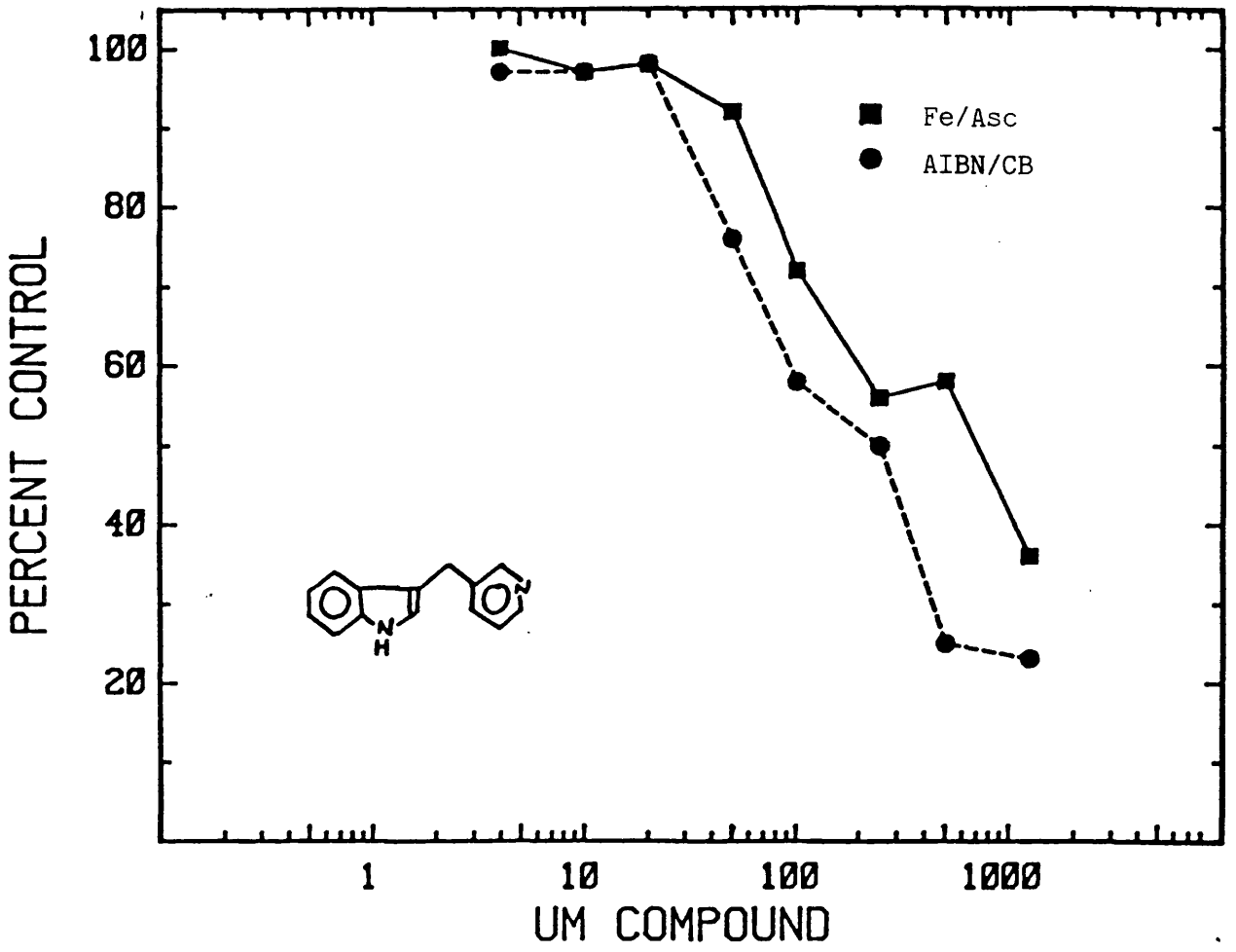
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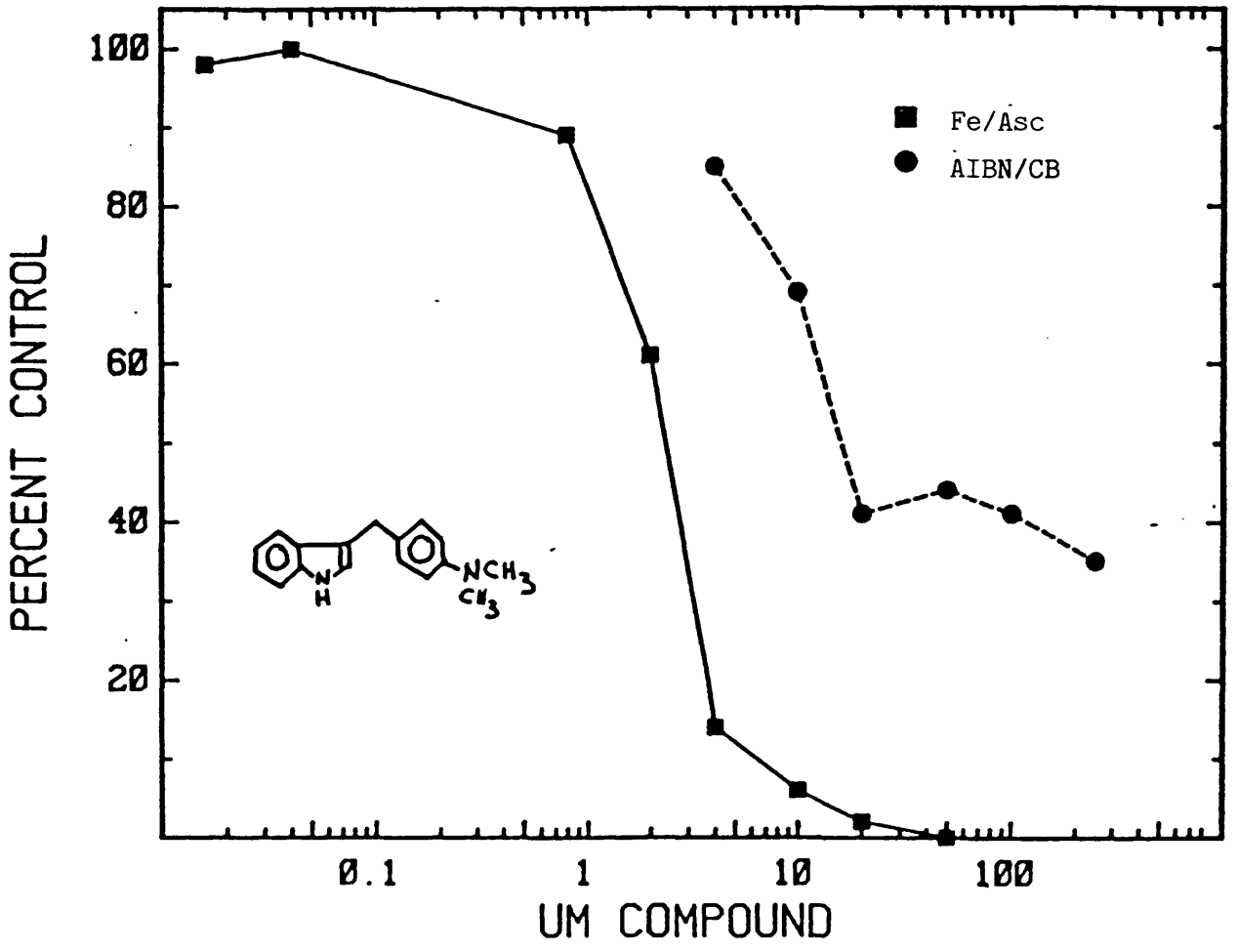
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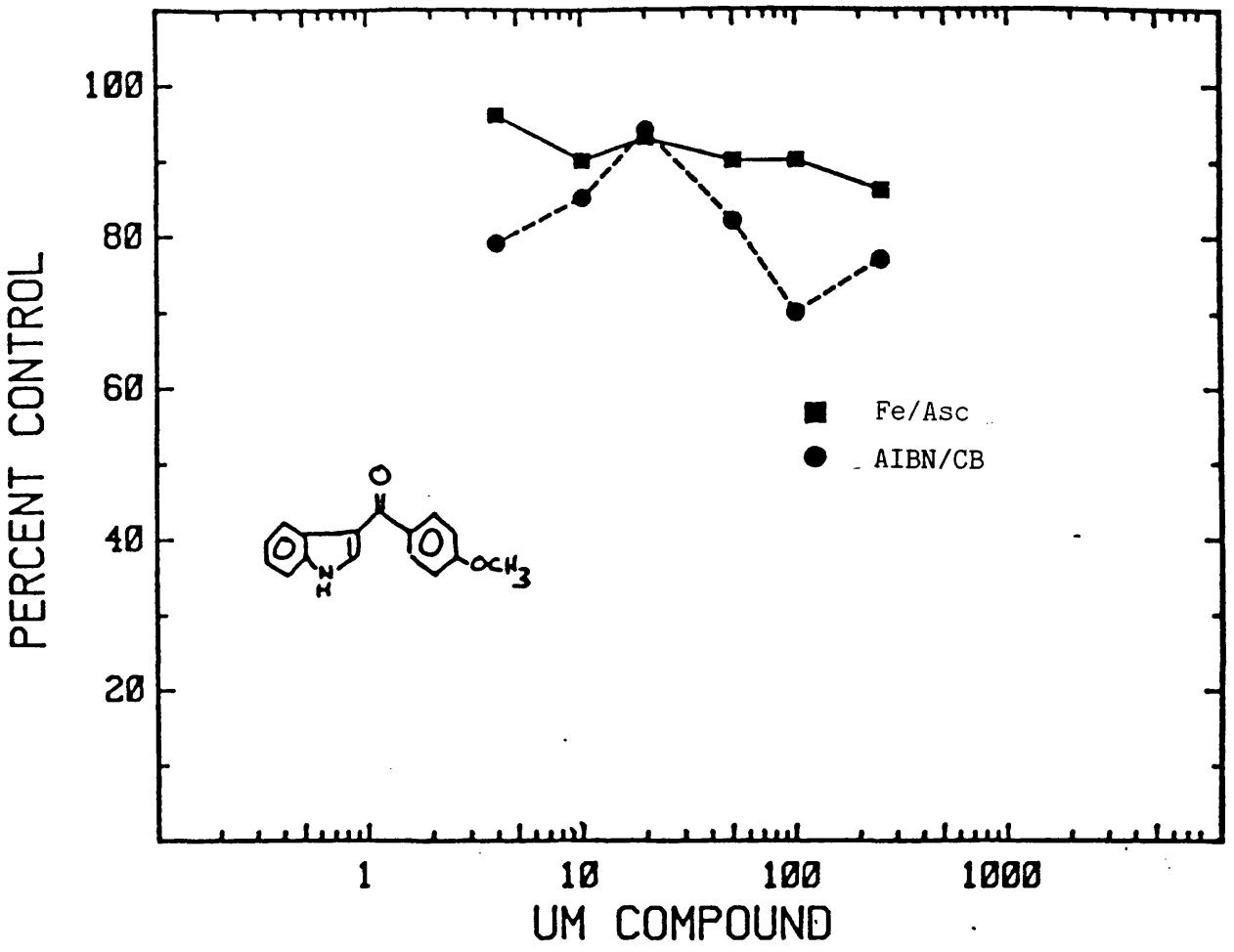
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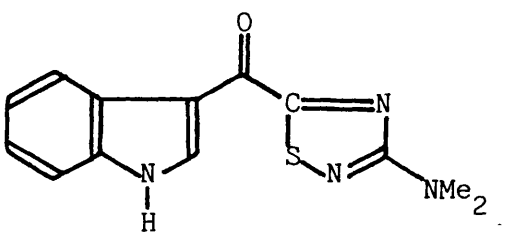
C H A P T E R 2

Synthesis of Dendrodoine

INTRODUCTION

Recently the isolation and structural determination of dendrodoine 5-[3-(N,N-dimethylamino)-1,2,4-thiadiazolyl]-3-indolylmethanone (61) has been reported[40]. This compound is a metabolite of the marine tunicate Dendroda grossular, indigenous to the north coast of Brittany, and it represents the first naturally occurring 1,2,4-thiadiazolyl derivative to be characterised.



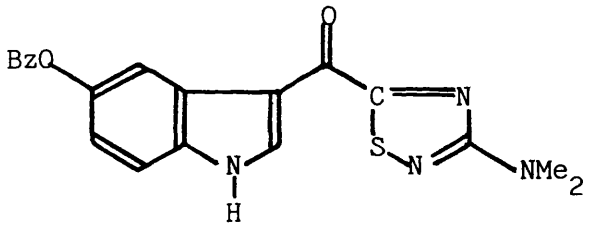
(61)

Interestingly, dendrodoine (61) was claimed to have anti-cancer activity, but unfortunately only a small amount (100mg from 2200kg of animal tissue) was available, and so these claims were based on cell culture systems and a token number of animal experiments.

Clearly a synthesis of the alkaloid was required in order to supply enough dendrodoine for a "proper" assessment of its pharmacology. In addition, the unusual structure needed to be checked, for, of course, ^{1}H nmr spectroscopy does not differentiate between the various isomeric arrangements of the thiadiazole ring.

DISCUSSION AND RESULTS

We originally set out to design a viable synthetic route to dendrodoine (61) to allow its biological properties to be fully investigated and, if this was successful, it was then hoped to prepare a variety of analogues of this compound. In particular the 5-benzyloxy derivative (62) and the methylene compound (63) seemed to be useful targets, for 5-hydroxyindoles often have an enhanced biological effect (see chapter 1) and the "methylene" derivative (63) might well have similar properties to the anti-oxidant structures discussed previously.



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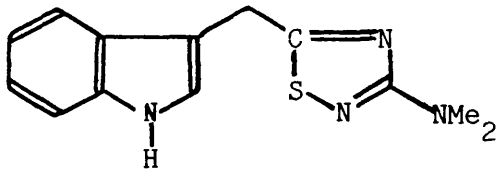
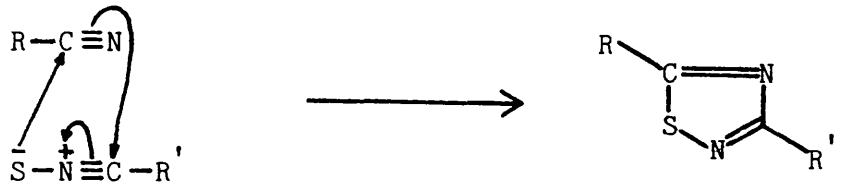


(63)

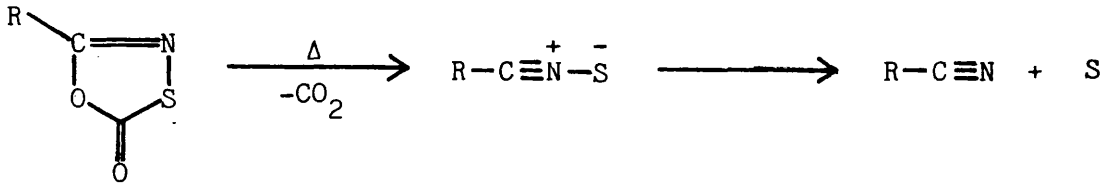
3,5-Disubstituted 1,2,4-thiadiazoles (64), where the two substituent groups R and R' are different (as is the case with dendrodoine (61)) can be generally prepared[41] by a 1,3-dipolar cycloaddition reaction of a nitrile sulphide (65) and a nitrile (Scheme 17). This strategy not only has the advantage of being regioselective with respect to the formation of the thiadiazole substituents but is also a convergent approach.



(65) (64)

SCHEME 17

Nitrile sulphides (65) are transient species which are usually generated in situ by the thermal decomposition of the corresponding 5-substituted 1,3,4-oxathiazol-2-one (66). They are prone to further degradation, however, leading to the nitrile and sulphur (Scheme 18).

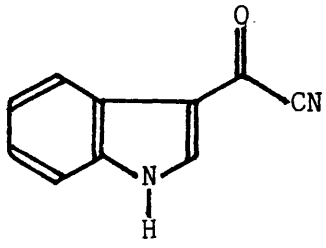


(66)

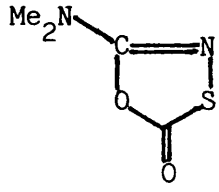
SCHEME 18

The cycloaddition reaction is carried out at high temperatures (usually circa 200°C) using a large excess of the nitrile (often the solvent[42,43]) in a high boiling non-polar medium. Decalin or dodecane can be used and, in general, non-polar solvents are recommended in order to prevent any partial charge neutralisation which may result from solvation of the reactive species. The oxathiazolone (66) is normally added very slowly or in aliquots over a period of time to the nitrile at the required temperature. The reason for this mode of addition is that the sulphur atoms, produced by the further decomposition of the nitrile sulphide form short sulphur chains which can attach themselves to sulphur atoms of the nitrile sulphide. This is said to enhance the rate of the degradation process. On the other hand, chain growth stops with the formation of stable S_8 rings. Thus it is recommended that time should elapse to enable this to take place before the next addition of oxathiazolone.

To synthesise dendrodoine (61) by this route, supplies of the nitrile (67) and the oxathiazolone (68) were required, neither of which had been previously described.



(67)

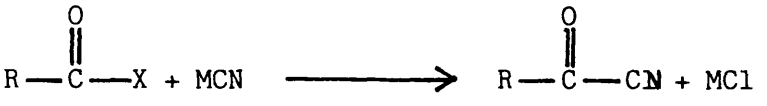


(68)

Common sense arguments predict, however, that the relatively electron poor nitrile (67) and the electron rich nitrile sulphide, produced from the oxathiazolone (68), should react well together and give the required addition product, dendrodoine (61).

Indole-3-carbonyl nitrile (67)

Acyl nitriles are generally prepared by the reaction of an acyl halide with a variety of metal cyanides[45] (Scheme 19).



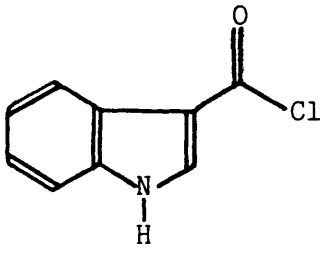
R = Alkyl or aryl

M = Na, K, Cu, Ti, Me_3Si, $\underline{n}$-Bu_3Sn

SCHEME 19

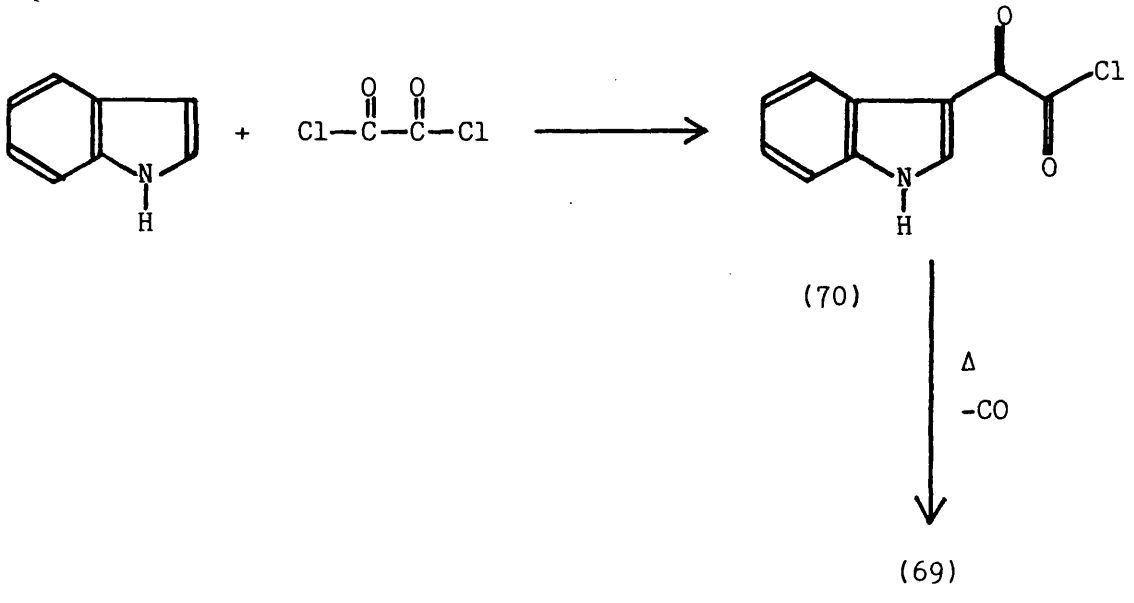
Acyl chlorides[45] are the usual organic substrates because of their availability, but bromides and iodides are sometimes employed[46].

Unfortunately the acyl chloride required in our planned route, namely indole-3-carbonyl chloride (69), is difficult to prepare, is relatively unstable and tends to form resins.



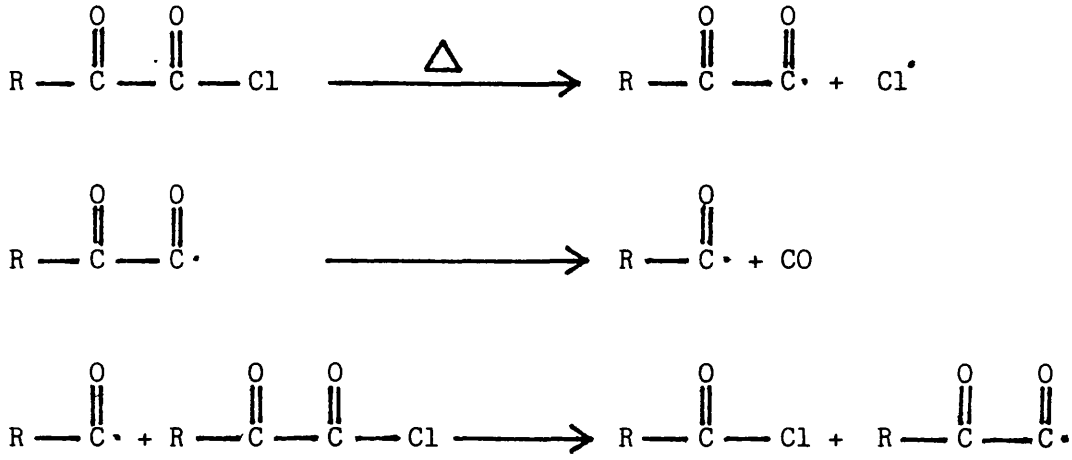
(69)

One of the few reported syntheses[47] of this compound involves the thermolysis of indole-3-glyoxylyl chloride (70). The temperature required is ~120°C and the starting material is itself formed by reacting indole with oxalyl chloride (Scheme 20).



SCHEME 20

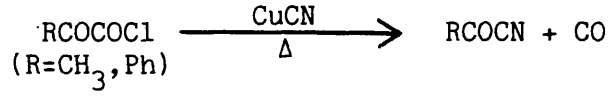
The thermolysis process is considered to proceed by a radical mechanism (Scheme 21).



SCHEME 21

However, the yield is poor (~ 20%) and much resin is formed. This fact caused us to speculate on ways to improve the route. For example, we argued that, if a transition metal cyanide were present during the decarbonylation step, then it might be possible to trap out the indolylcarbonyl radical as the indolylcarbonyl nitrile (67). If this compound were less reactive (more stable) than the corresponding chloride, an increased productivity would be assured.

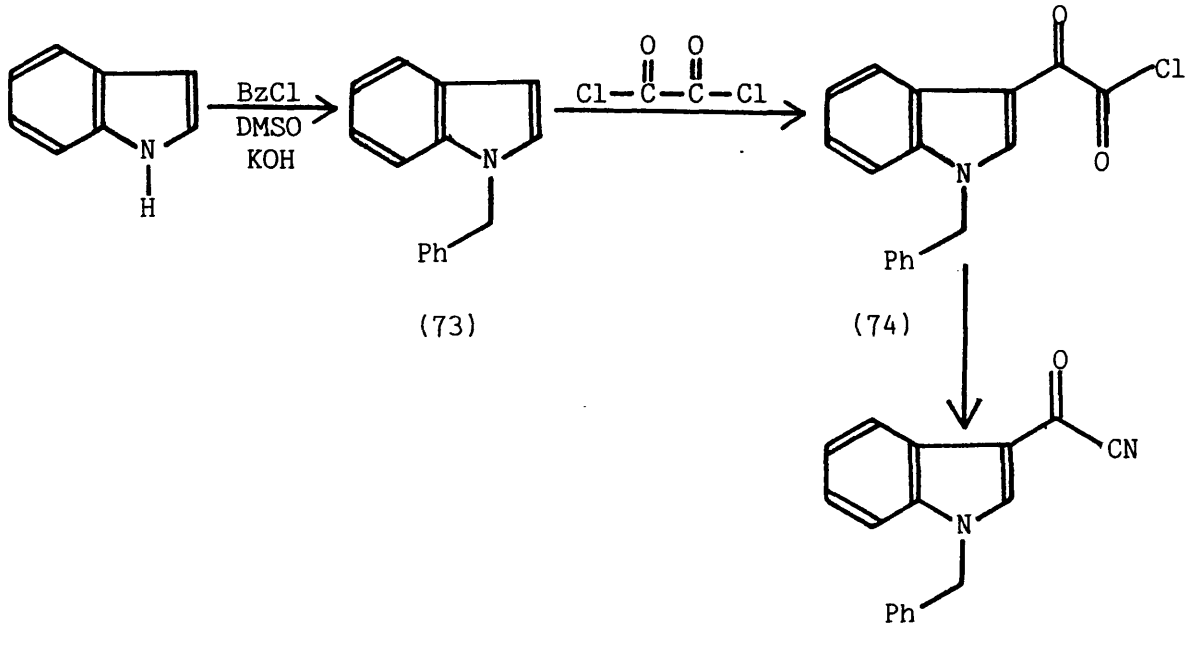
A survey of the literature revealed that acyl nitriles had indeed been made this way, albeit by accident. Tanner and Das[47] were attempting to synthesise glyoxylyl nitriles by heating glyoxylyl chlorides (71) with copper (I) cyanide in chlorobenzene at the boiling point. To their surprise, the corresponding acyl nitriles (72) formed instead. (Scheme 22)



(71) (72)

SCHEME 22

We then set about trying this type of approach on the indolyl analogue, but at first we selected a N-protected indole in order to preclude potential polymerisation at this position. Thus 1-benzyl-indole (73), prepared from indole and benzyl bromide, was reacted with oxalyl chloride to afford the desired 3-indolyl derivative (74) as a yellow solid (81% yield). This was then heated[48] in boiling toluene with acetonitrile and copper (I) cyanide to yield on work-up the corresponding acyl nitrile in 69% yield as a crystalline colourless solid (Scheme 23).

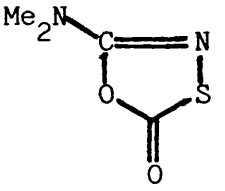


(73) (74) (75)

SCHEME 23

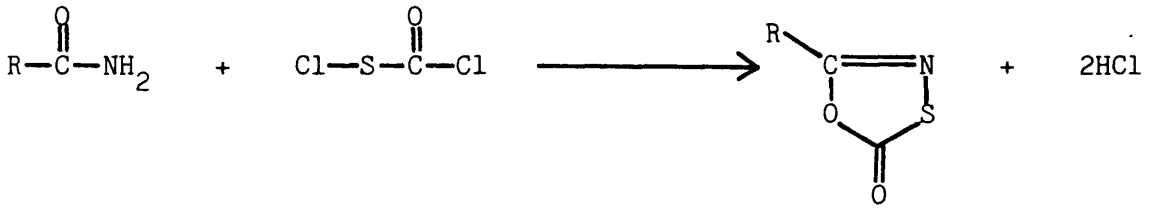
Things had gone so well that we next sought to remove the added complications arising from protection/deprotection of the indolic nitrogen atom, and we were able to show that this was unnecessary, for when indole was used directly the same sequence gave firstly the glyoxylyl chloride (70) in 82% yield, and then the acyl nitrile (67) in 52%. Further experiments proved that it was possible to leave out the purification of the glyoxylyl chloride intermediate and simply heat the acylation mixture with added copper (I) cyanide and the solvent mixture.

5-(N,N-Dimethylamino)-1,3,4-oxathiazol-2-one (68)



(68)

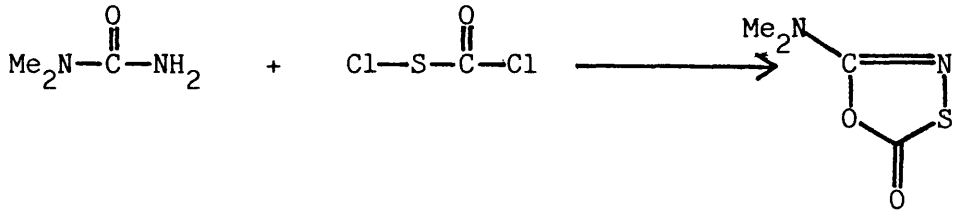
1,3,4-Oxathiazol-2-ones (66) are generally prepared[50] by reaction of the corresponding amide with chlorocarbonylsulphenyl chloride (76) (Scheme 24).



(76) (66)

SCHEME 24

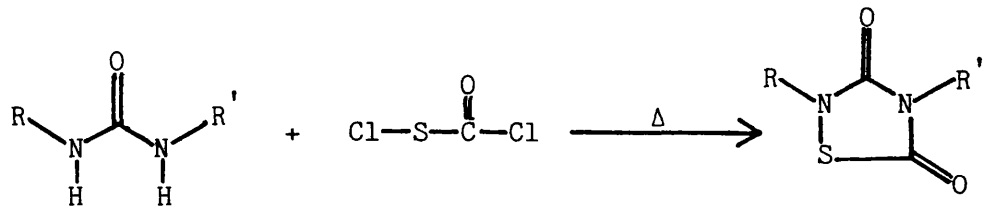
The reaction is carried out by heating the components in an inert solvent, usually toluene. This strategy has been used successfully to synthesise a wide range of both alkyl and aryl 1,3,4-oxathiazol-2-ones. To prepare our target compound (68) by this route, the coupling of 1,1-dimethylurea (77) with chlorocarbonylsulphenyl chloride (76) is required (Scheme 25).



(77) (76) (68)

SCHEME 25

Although reactions of 1,1-disubstituted ureas with chlorocarbonylsulphenyl chloride have not been previously reported, a considerable amount of work has been carried out using 1,3-disubstituted ureas (78). For example, 1,3-disubstitued ureas (78) react[51,52] with chlorocarbonylsulphenyl chloride at both nitrogen centres to yield disubstituted thiaimidazolidines (79) (Scheme 26), which are used commercially as herbicides.

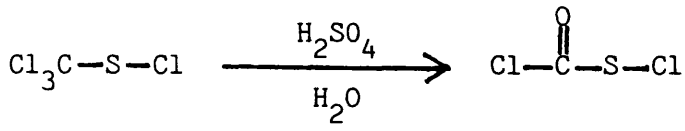


(78) (76) (79)

SCHEME 26

It is therefore apparent that ureas will undergo cycloaddition reaction with chlorocarbonylsulphenyl chloride (76) and as 1,1-dimethylurea (77) can only react through one of its nitrogen centres, there would seem no apparent reason why our proposed reaction should not yield the required 1,3,4-oxathiazol-2-one (68).

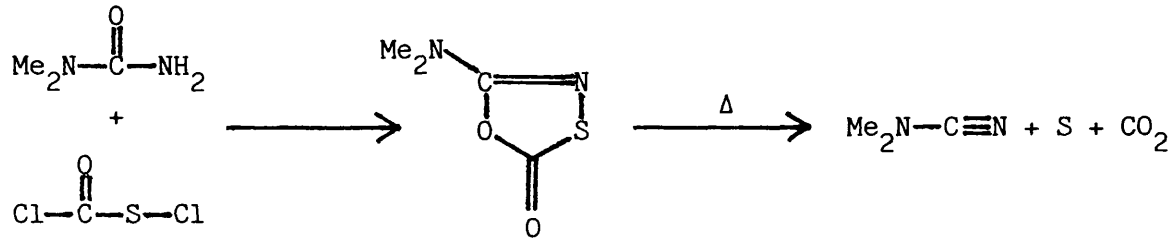
Chlorocarbonylsulphenyl chloride (76) is commercially available, but very expensive and unstable, and is therefore best prepared[53] as and when required, by the acid catalysed part-hydrolysis of trichloromethylsulphenyl chloride (80) (Scheme 27).



(80) (76)

SCHEME 27

1,1-Dimethylurea (77) stirred in toluene was treated with one equivalent of chlorocarbonylsulphenyl chloride (76) and the resultant mixture heated to reflux for several hours. At the end of this time, a large amount of sulphur had separated out. None of the desired oxathiazolone was obtained, but the toluene phase did contain dimethylaminonitrile (81). Thus it was clear that these conditions were too harsh and that although the correct product formed, it also underwent thermolysis as shown in Scheme 28.



(67) (81)

SCHEME 28

The reaction was then repeated using the same solvents, but at room temperature. Regular TLC analysis of the reaction mixture indicated the slow but steady formation of a new product spot. After 15 hours the reaction was stopped and the product purified by chromatography to yield 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68) as an orange liquid (11%). Its thermal degradation was monitored by ^{1}H nmr spectroscopy and the compound was observed to decompose to dimethylaminonitrile at 60° - 70°C.

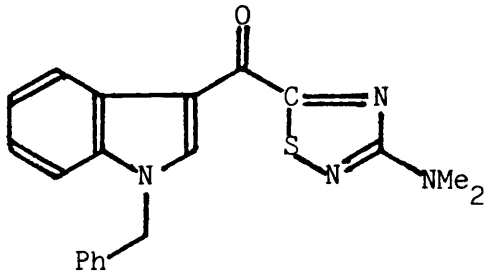
The yield of heterocycle produced was disappointing but in cold toluene N,N-dimethylurea shows only limited solubility. Thus a better solvent was required. In addition, since hydrogen chloride is liberated in the reaction, it is possible that some deactivation of the starting material is effected by protonation.

Addition of pyridine to the medium caused a violent reaction, so the latter problem was overcome by adding a 3-4 fold excess of N,N-dimethylurea. Under these conditions and in acetonitrile rather than toluene, the yield of 1,3,4-oxathiazol-2-one was increased to 63% - 75%. Unused amide could easily be recovered.

As a preliminary run, the N-benzylated acyl nitrile (75) was heated to 195°C and the oxathiazolone gradually added. A large excess of the nitrile was used (25-fold), itself acting as the solvent and on work-up the benzyl derivative of dendrodoine (82) was isolated in 78% yield (based on the oxathiazolone). Using a lower ratio of the acyl nitrile (4-fold) in decalin gave a reduced yield (10%).

To be efficient this approach should not rely on large excesses of reagents, and in fact we observed that, if the acyl nitrile in the minimum of decalin was heated with an 1.2 molar amount of the oxathiazolone

(added in portions), the yield was only slightly less (~ 70%) than in the first attempt described above.

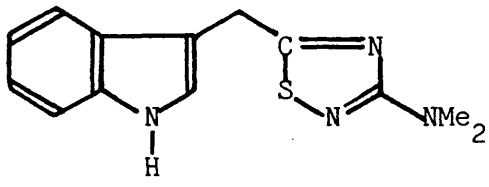


(82)

Our choice of the benzylated acyl nitrile (75) rather than the obvious one of the direct precursor (67) of dendrodoine itself, was caused by the fact that the latter acyl nitrile decomposes at 190°C, and indeed repetitions of the last procedure now at 185°C with this substrate failed to give dendrodoine. However, using dimethylformamide in which the acyl nitrile was more soluble than decalin, 1.2 molar excess of the oxathiazolone (68) and a reaction temperature of 145°C, a product was obtained which proved to be identical in every respect to the natural product dendrodoine (30% yield). The isolation of this compound was somewhat hampered by the fact that unchanged acyl nitrile had very similar retention indices in most solvents on silica or alumina. However, this problem was soon solved by changing to reverse phase techniques (see experimental section).

Now that the natural product had been synthesised, we turned our attention to the preparation of the interesting analogues of this compound previously discussed.

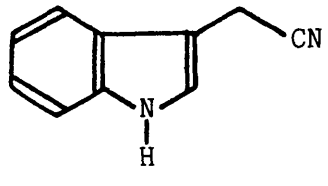
3-(N,N-Dimethylamino)-5-(indole-3-methylene)-1,2,4-thiadiazole (63)



(63)

This compound (63) was of particular interest to us, because of the work we were carrying out on indole-3-methylene inhibitors. It was also considered to be a possible metabolic precursor to dendrodoine (61) itself.

The first approach to this compound was the cycloaddition of the oxathiazolone (68) and indole-3-acetonitrile (6).

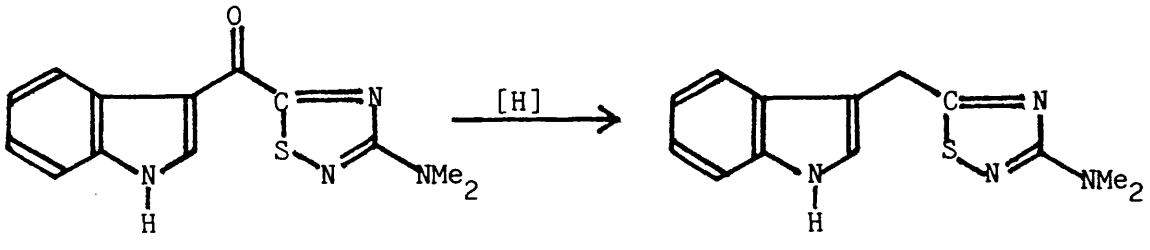


(6)

The reaction was carried out at first using decalin and dimethylformamide as solvents and up to 5-molar equivalents of the oxathiazolone. No addition products were isolated, however, in experiments conducted over a range of temperatures. Other solvents were then employed:- p-xylene (140°C), chlorobenzene (130°C), m-dichlorobenzene (180°C), diglyme (150°C), p-nitrotoluene (180°C), methylformamide (180°C) and dimethyl sulphoxide (180°C). In each case the result was the same and the nitrile was recovered unchanged.

Although the failure of this reaction was disappointing, it was not completely unexpected, as the absence of the carbonyl function (in compound (6)) would greatly reduce the electrophilicity of the nitrile group and consequently the potency as a 1,3-dipolarphile.

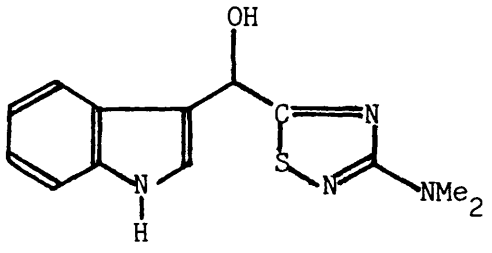
Next we attempted to prepare our target compound (63) by reduction of the parent ketone (61) (Scheme 29).



(61) (63)

SCHEME 29

This reaction was first attempted on a small scale using sodium borohydride in methanol at room temperature. However, after one hour, no reaction had occurred, so the mixture was then heated to reflux and the starting material was seen to steadily be converted to a slightly more polar compound. This new compound was presumed to be the alcohol (84).



(84)

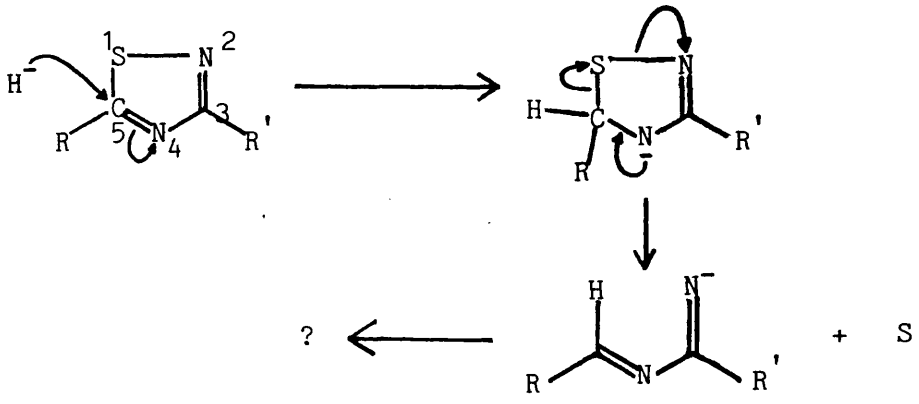
Complete conversion to this new compound took approximately one hour. As no further change was seen after a second hour at reflux, the reaction was worked up and the product purified by column chromatography. Although a mass spectrometric analysis of this compound suggested it to be the expected alcohol (84), the yield only accounted for 5% of the starting material and insufficient was available for further useful characterisation.

It was hoped that, if the reductant was changed to lithium aluminium hydride, the ketone would be converted directly to the required methylene compound (63) in improved yield. Thus, dedrodoine (61) was added to a suspension of lithium aluminium hydride in dry tetrahydrofuran at -78°C and stirred for one hour. As no reaction occurred after this

time, the mixture was then allowed to warm up, 10°C at a time with one hour intervals in between. At -40°C a slow conversion of the starting material to the previous product began. On reaching room termperature, all the dendrodoine (61) had reacted to yield the previous product (84). Stirring for several more hours at room temperature again failed to convert this compound any further, so the reaction was worked up and the product purified by column chromatography. Once again, however, the product accounted for less than 5% of the starting material (61).

Several other reduction systems, namely diborane, Wolff Kishner and sodium cyanoborohydride were also used. The first two produced similar results as above and the last effected no reaction at all.

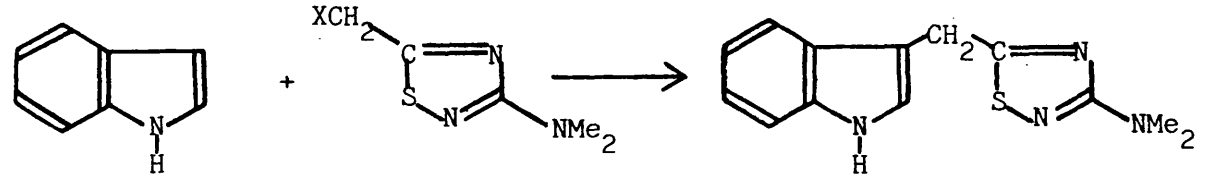
The failure of this approach is probably due to reductive cleavage of the 1,2,4-thiadiazole ring by initial attack of hydride ion, or its equivalent, at the electrophilic C-5 carbon (Scheme 30).



SCHEME 30

Although substitution of thiadiazole rings generally[41b] increases their stability towards reducing agents in our compound (61), the presence of the carbonyl substituent on the 5-position of the ring probably increases the electrophilicity of the 5-carbon atom and hence facilitates breakdown.

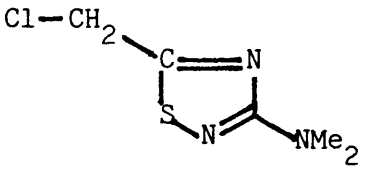
Finally we considered a completely different approach to the target compound (63) in which a 5-halomethylthiadiazole (85) was prepared first and subsequently reacted with indole or the indolyl anion (Scheme 31).



(85)

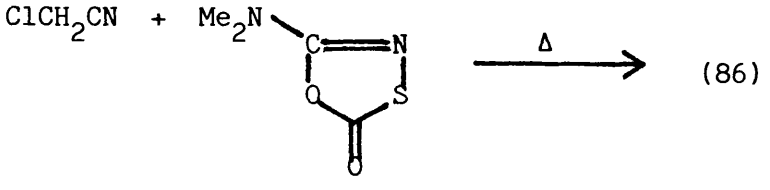
SCHEME 31

The route, if successful, would not only give a viable synthesis of the target compound (63), but also allow the preparation of numerous other compounds containing the unusual 3-(N,N-dimethylamino)-1,2,4-thiadiazole moiety. It was therefore decided to attempt a synthesis of 5-chloromethyl-3-(N,N-dimethylamino)-1,2,4-thiadiazole (86).



(86)

If this compound was to be prepared by the usual route, it would require the cycloaddition of chloroacetonitrile (87) and 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68) (Scheme 32).

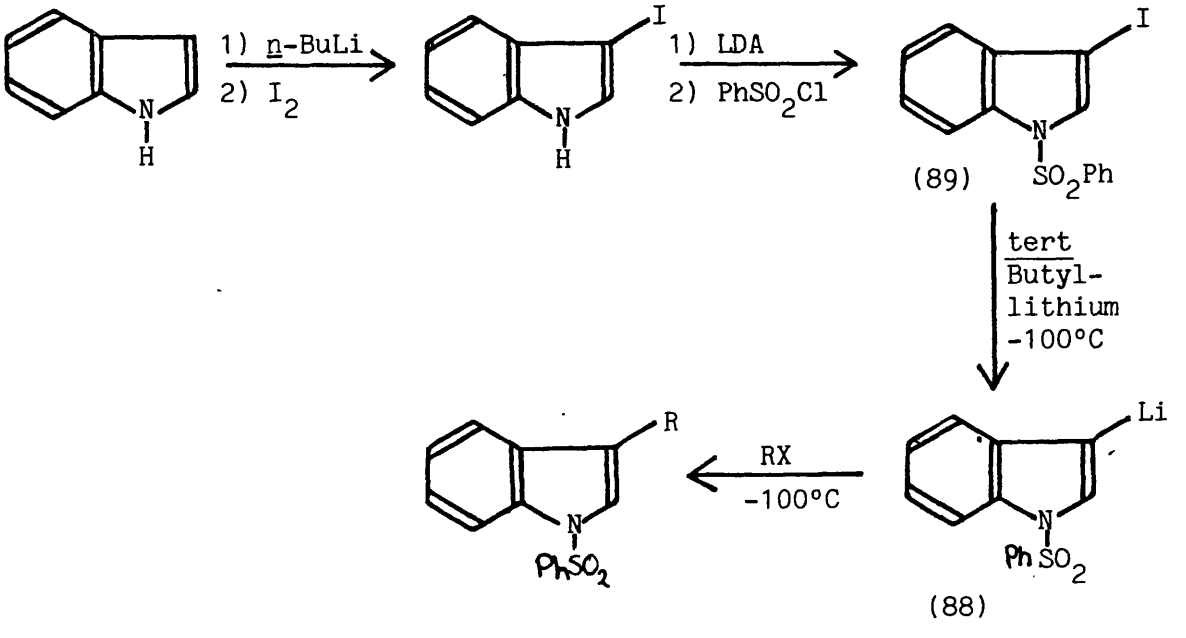


(87) (68)

SCHEME 32

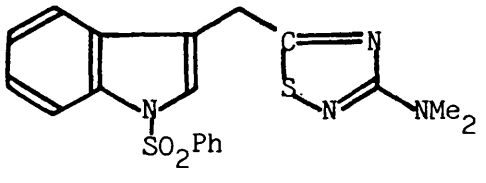
Due to the lower reactivity of alkyl nitriles (vs aryl nitriles) to nucleophilic attack, they cannot be generally employed in the synthesis of 1,2,4-thiadiazoles. In fact, only the use of one such compound has been reported[54], namely ethyl cyanoacetate(136a), and here the electron withdrawing ethoxycarbonyl group plays an important role in promoting the reaction.

We wondered if the chlorine atom would enhance the reactivity of our substrate (87), by an inductive mechanism, and the reaction was first tried using an excess of the nitrile (87) as solvent at 120°C. No products formed and so it was repeated using a ten fold excess of the nitrile (87) in decalin as solvent at 160°C. After work-up the reaction yielded the required 5-(chloromethyl)-3-(N,N-dimethylamino)-1,2,4-thiadiazole (86) in reasonable yield (50%). The second part of the synthesis was then attempted using indole magnesium bromide in dry tetrahydrofuran as reactant. Disappointingly, after stirring at room temperature for 15 hours, no reaction occurred, so the mixture was then heated at reflux for several hours. Still no products formed and the reaction was then repeated using 3-lithio-1-(phenylsulphonyl)indole (88) in the hope that this "harder" nucleophile would effect the reaction we required. 3-Lithio-1-(phenylsulphonyl)indole (88) was first prepared by Saulnier and Gribble[55] from 3-iodo-1-(phenylsulphonyl)indole (89) at -100°C (Scheme 33) with tert-butyllithium and reacted successfully at this temperature with a wide range of electrophiles.

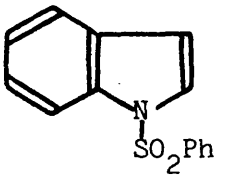


SCHEME 33

5-(Chloromethyl)-3-(N,N-dimethylamino)-1,2,4-thiadiazole (86) was reacted with 3-lithio-(1-phenylsulphonyl)indole (88), generated by the literature procedure, at -100°C. TLC analysis of the subsequent reaction mixture showed a single product to be present with an associated loss of the thiadiazole (86). On work-up, however, the product of this reaction was not the expected thiadiazole (90) but simply 1-(phenyl-sulphonyl)indole (91).

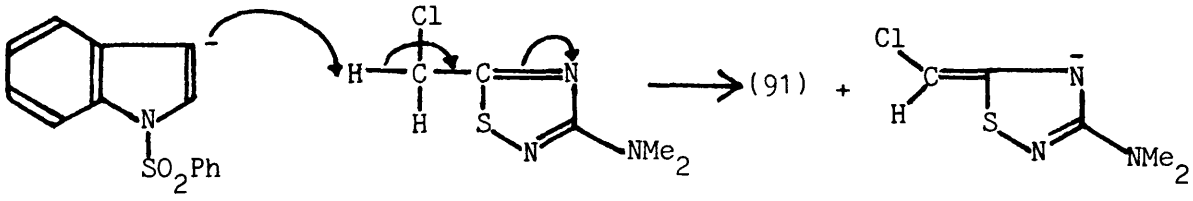


(90)



(91)

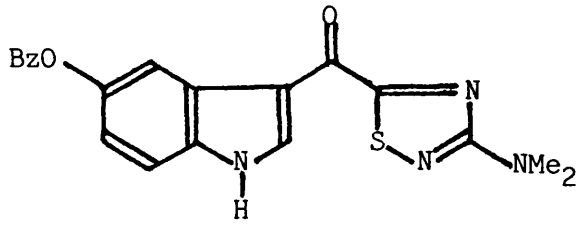
Although this compound (91) would have resulted on work-up if the 3-lithio derivative (88) had not reacted, the loss of the thiadiazole (86) from the reaction mixture tends to suggest that proton extraction has occurred, probably from the methylene group of the oxathiazole as shown (Scheme 34). However, the fate of the oxathiazole anion remains unknown.



SCHEME 34

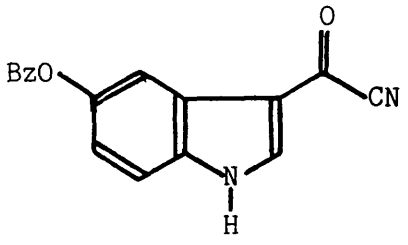
As all the readily available routes to this compound (63) had now been investigated and shown to fail, work on this compound was suspended.

5-[3-(N,N-Dimethylamino)-1,2,4-thiadiazolyl]-3-(5-benzyloxyindolyl)-methanone (62)



(62)

The synthesis of the target compound via the previously employed cycloaddition process requires the presynthesis of 5-benzyloxyindole-3-carbonyl nitrile (92).



(92)

5-Benzyloxyindole (54) was reacted with oxalyl chloride to yield the required glyoxylyl chloride (93) in quantitative yield. The first attempt to convert this compound (93) to the required acyl nitrile (92) using the method previously employed and a reaction time of six hours only yielded 23% of the required product (92). In an attempt to improve the yield of this reaction, the effect of reaction time and temperature were studied by repeating the reaction on a small scale using acetonitrile, chlorobenzene and toluene as solvents at reflux for various reaction times. From the results of these experiments, an optimum yield (71%) of 5-benzyloxyindole-3-carbonyl nitrile (92) was obtained when toluene was used as solvent with a reaction time of two hours. The reaction was repeated on a larger scale using these optimum conditions to yield 67% of the required acyl nitrile (92).

Conversion of this acyl nitrile (92) to the corresponding dimethylaminothiadiazole (62) was first attempted by the standard conditions

using decalin as solvent at 190°C. Even after the portion wise addition of 3 molar equivalents of the oxathiazolone (68), however, TLC analysis showed that no reaction had occurred, and the resultant work-up and purification of the reaction mixture yielded one equivalent of sulphur (cf oxathiazolone (68)) and the starting acyl nitrile (92) untouched. The reaction was then repeated using the various solvent and temperature combinations used previously in attempts to synthesise the indole-3-methylene derivative (63). In all these systems, however, no reaction occurred. Finally, the reaction was tried using an excess of the molten nitrile (~240°C) and no solvent. Unfortunately, however, the high temperature involved caused the decomposition of the acyl nitrile (92).

Once again the failure of this cyclisation reaction can probably be explained by a reduction in the electrophilicity of the reacting nitrile (92), in this case caused by the introduction of an electron donating substituent onto the indole nucleus.

EXPERIMENTAL

Route to 1-benzylindole-3-carbonyl nitrile (75)

a) 1-Benzylindole (73)[49]

Crushed potassium hydroxide (22.4g, 0.4mol) was stirred at room temperature in dry distilled DMSO ($200cm^3$) for one hour, before indole (11.7g, 0.1mol) was added. After a further 45 minutes, the solution was cooled in an ice bath and benzyl bromide (34.2g, 0.2mol), added dropwise. The ice bath was then removed and the reaction mixture stirred for 45 minutes, then quenched with water ($200cm^3$). The resultant aqueous suspension was extracted with diethyl ether (3 x $100cm^3$), with each individual ether fraction being washed with water (3 x $50cm^3$). The organic fractions were then combined, dried ($MgSO_4$) and the solvent and excess benzyl bromide removed under reduced pressure to yield a colourless solid (73) (17.5g, 85%), crystallised from ethanol, m.p. 44-45°C (lit[50], 43°C).

b) 1-Benzylindole-3-glyoxylyl chloride (74)

To a solution of 1-benzylindole (73) (16g, 77mmol) in dry diethyl ether ($200cm^3$), vigorously stirred and cooled in an ice bath was added, dropwise, freshly distilled oxyalyl chloride ($7cm^3$, 80mmol). After addition, which caused the steady precipitation of a yellow solid, the reaction mixture was stirred for a further 2 hours, then the yellow precipitate filtered under nitrogen and washed with dry diethyl ether ($50cm^3$) to yield a yellow solid (74) (18.5g, 81%), which on exposure to air steadily turned orange, m.p. 95-96°C (dec).

I.R. - ν_{max}(Nujol)cm^{-1}; 1785(COCOCl), 1645(COCOCl).

M.S. - (low eV, EI)m/z; 297(4%,[M^+]), 269(8%), 234(100%), 91(32%).

c) 1-Benzylindole-3-carbonyl nitrile (75)

The chloride (74) (52g,17.5mmol), copper I cyanide (3g,33mmol), distilled acetonitrile (1.5cm^3, 29mmol) and dry toluene (50cm^3) were heated to reflux with stirring under dry conditions for three hours. After this time, the reaction mixture was filtered hot and the solid residue washed with hot toluene (2 x 25cm^3). The toluene fractions were combined, boiled with activated charcoal for five minutes, then filtered and the solvent removed to yield 1-benzylindole-3-carbonyl nitrile (75)(3.2g,69%), crystallised from toluene/petroleum in ether, m.p. 181-182°C.

Elemental analysis - $C_{17}H_{12}N_2O$; Requires - C,78.44: H,4.65: N,10.76.
Found - C,78.43: H,4.64: N,10.68.

U.V. - λ_{max}(Methanol)nm(ε); 338(14700), 288(13730), 221(23030).

I.R. - ν_{max}(Nujol)cm^{-1}; 2210(C≡N), 1630(C=O).

M.S. - (low eV, EI)m/z; 260(100%,[M^+]), 91(51%).

N.M.R. - δ_H(d^6-DMSO)ppm; 8.64(1H,s,indole H-2), 8.16-8.04(1H,m,indole H-4), 7.72-7.14(8H,m,rest of aromatic protons), 5.66(2H,s,CH_2).

- δ_C(d^6-DMSO)ppm; 152.2(s,C=O), 143.4(s,C-1'), 137.4(s,C-8), 135.8(d), 128.7(d), 127.9+127.4+124.9(3d,phenyl C-H), 124.1(s,C-9), 121.3(d), 115.6+114.7(2s,C-3,C≡N), 112.3(d,C-7), 50.4(t,CH_2).

Subsequently, this reaction was repeated without isolation and purification of the glyoxylyl chloride (74). The diethyl ether was simply removed, under reduced pressure, and the copper I cyanide, acetonitrile and toluene added to the crude solid, then heated as before. By this method, the efficiency of the reaction was improved to yield the acyl nitrile (75) in 49% overall yield from indole.

Route to Indole-3-carbonyl nitrile (67)

a) Indole-3-glyoxylyl chloride (70)

Indole (10g, 85mmol) was readily converted by the above method to its glyoxylyl chloride (70) (14.6g, 82%), a yellow crystalline solid.

I.R. - ν_{max}(Nujol)cm^{-1}; 3300(NH), 1755(COCOCl), 1620(COCOCl).

M.S. - (70 eV, EI)m/z; 207(5%,[M^+]), 144(100%), 143(53%), 115(29%).

b) Indole-3-carbonyl nitrile (67)

Under the same conditions as employed for the benzyl derivative (74) indole-3-glyoxylyl chloride (70) (5g, 24mmol) was heated for three hours with copper I cyanide (4g, 42mmol), acetonitrile ($2.2cm^3$, 42mmol) in dry toluene ($50cm^3$), to yield, after chromatographic separation, the required acyl nitrile (67) (2.2g, 52%), as a colourless solid which was crystallised from an ethyl acetate/petroleum ether mixture, m.p. 190°C(dec).

Elemental Analysis - $C_{10}H_6N_2O$; Requires - C,70.60: H,3.55: N,16.50

Found - C,71.00: H,3.40: N,16.30.

I.R. - ν_{max}(Nujol)cm^{-1}; 3200(N-H), 2200(very weak,C≡N), 1615(C=O).

M.S. - (low eV, EI)m/z; 170[M^+].

- (70 eV, EI)m/z; 170(77%,[M^+]), 144(100%).

N.M.R. - δ_H(d^6-DMSO)ppm; 13.11-12.71(1H,br.s,N-H), 9.60(1H,s,H-2), 8.05(1H,m,H-4), 7.82(1H,m,H-7), 7.20(2H,m,H-5,H-6).

- δ_C($(CD_3)_2CO$)ppm; 158.6(s,C=O), 137.6(s,C-8), 124.8(d), 124.3(s,C-9), 123.7(d), 121.0(d), 116.3+114.3(2s,C-3,C≡N), 113.3(d).

U.V. - λ_{max}(ε)nm; 338(11540), 273(10390), 266(10600), 255(10875), 212(13220).

Repeating this reaction without isolating the glyoxylyl chloride (70) also improved the yield of the nitrile (67) to 52% from indole.

Route to 5-benzyloxyindole-3-carbonyl nitrile (92)

a) 5-Benzyloxyindole-3-glyoxylyl chloride (93)

5-Benzyloxyindole (54) (1g, 4.5mmol) was reacted with oxalyl chloride ($0.5cm^3$, 5.7mmol) in dry diethyl ether ($10cm^3$) as above, to yield a yellow crystalline solid (93) (1.4g, 100%), m.p. 151-152°C (dec).

I.R. - ν_{max}(Nujol)cm^{-1}; 3200(N-H), 1790(CO<u>CO</u>Cl), 1630(<u>CO</u>COCl).

b) 5-Benzyloxyindole-3-carbonyl nitrile (92)

The glyoxylyl chloride (93) was then heated as before with copper I cyanide, acetonitrile and toluene to yield, after six hours, on work up and chromatographic separation, 23% of the required acyl nitrile (92). Because of the disappointingly low yield of this reaction, it was repeated on a 100mg of the glyoxylyl chloride (93), using acetonitrile, toluene and chlorobenzene as solvents, at reflux, over various reaction times:-

Acetonitrile - 15 hours, no product.
Toluene - 30 minutes, 40%; 1 hour, 46%; 2 hours, 74%; 3 hours, 59%; 15 hours, no product.
Chlorobenzene - 45 minutes, 63%; 2 hours, 50%; 3 hours, 50%; 15 hours, no product.

The acyl nitrile (92) was crystallised from ethyl acetate/petroleum ether, m.p. 224-226°C.

Elemental analysis - $C_{17}H_{12}N_2O_2$; Requires - C,73.90: H,4.38: N,10.14.

Found - C,73.76: H,4.20: N, 9.77.

U.V. - λ_{max}(Methanol)nm(ε); 336(7000), 291(10640), 252(11560), 220(28120).

I.R. - ν_{max}(Nujol)cm^{-1}; 3200(N-H), 2220(very weak,C≡N), 1617(C=O).

M.S. - (low eV, EI)m/z; 276(88%,[M^+]), 159(43%), 91(100%).

N.M.R. - δ_H(d^6-DMSO)ppm; 12.76(1H,br.s,N-H), 8.53(1H,s,H-2), 7.73-7.32 (7H,m,benzyl aromatics and H-4,H-7), 7.16-7.04(1H,m,H-6), 5.16(2H,s,CH_2).

- δ_C(d^6-DMSO)ppm; 158.3(s,C=O), 155.9(s,C-5), 140.7+137.0(2s,C-1', C-8), 132(s), 128.4+127.7+127.5(3d,phenyl C-H), 125.2(s) 116.2(s), 114.3(d), 114.1(d), 104.6(d), 69.7(t,CH_2).

Route to 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68)

a) Chlorocarbonylsulphenyl chloride (76)

Trichloromethylsulphenyl chloride (80) ($22cm^3$, 0.2mol) was added to concentrated sulphuric acid ($45cm^3$), containing water ($3.6cm^3$, 0.2mol). The two phase system was then heated to 50°C with rapid stirring, resulting in the vigorous evolution of hydrogen chloride gas. After one hour the rate of evolution had slowed dramatically, so the light layer was decanted off and distilled under nitrogen to yield a yellow oil (76) (12g, 46%), b.p. 98°C (lit[53], 98°C).

b) 5-(N,N-Dimethylamino)-1,3,4-oxathiazol-2-one (68)

To a stirred suspension/solution of 1,1-dimethylurea (77) (25g, 0.3mol) in dry distilled acetonitrile ($200cm^3$) under a nitrogen atmosphere

was added, dropwise, a solution of chlorocarbonylsulphenyl chloride (76) (11.5g, 0.09mol) in acetonitrile ($20cm^3$). The reaction mixture was stirred for one hour and then methanol ($10cm^3$) was added to decompose any traces of chlorocarbonylsulphenyl chloride, before filtration. The solid residue was recrystallised from ethanol to yield 1,1-dimethylurea (77) (15g). The filtrate was evaporated under reduced pressure, taking care to keep the temperature below 40°C, and purified by column chromatography to yield a yellow oil (68) (9.6g, 75%). When this reaction was repeated, yields of 63-75% were obtained.

U.V. - λ_{max}(Methanol)nm; 213

I.R. - ν_{max}(thin film)cm^{-1}; 1760(C=0), 1630(C=N).

M.S. - (low eV, EI)m/z; 146(50%,[M^+]), 72(100%).

N.M.R. - δ_H($CDCl_3$)ppm; 2.97(s,$N(C\underline{H}_3)_2$).

On heating the ^1H N.M.R. sample to above 60°C, the signal at 2.97ppm steadily converted to a singlet at 2.82ppm ($(C\underline{H}_3)_2N-C\equiv N$).

1-Benzyldendrodoine (82)

To a stirred solution of 1-benzylindole-3-carbonyl nitrile (75) (300mg, 1.15mmol) in decalin ($3cm^3$) at 190°C under a nitrogen atmosphere was added 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68) (200mg, 1.4mmol) at a rate of one drop every 20 seconds. After addition, the reaction mixture was stirred for a further five minutes, then the solvent removed and the resultant gum purified by column chromatography, to yield a yellow solid (82) (295mg, 71%) crystallised from dichloromethane/petroleum ether m.p. 186-187°C.

U.V. - λ_{max}(Methanol)nm(ε); 365(9550), 267(14150), 215(83370).

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 1605(C=O).

M.S. - (low eV, EI)m/z; 362(100%,[M^+]), 234(6%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 8.58(1H,s,H-2), 8.48-8.32(1H,m,H-4), 7.45-7.08(8H,m,rest of aromatic protons), 5.17(2H,s,$C\underline{H}_2Ph$), 3.02(6H,s,$N(C\underline{H}_3)_2$).

- $\delta_C(CDCl_3)$ppm; 188.0(s,C=O), 183.4+172.6(2s,thiadiazole C-3', C-5'), 139.5(d), 137.2(s,C-8), 134.2(s), 129.2+128.4+128.0 (3d,phenyl C-H), 126.4(s), 124.1(d), 123.5(d), 122.9(d), 113.1(s), 110.4(d), 51.1(t,$C\underline{H}_2Ph$), 38.9(1,$N(C\underline{H}_3)_2$).

Dendrodoine (61)

To a stirred solution of indole-3-carbonyl nitrile (67) (500mg, 3mmol) in dry distilled DMF ($1cm^3$) at 140°C under a nitrogen atmosphere was added 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68) (525mg, 3.6mmol) as before. After addition, the solvent was removed and the residue purified using reverse phase silica and a methanol/water solvent system, to yield dendrodoine (61) (240mg, 30%) as a yellow solid, crystallised from ethyl acetate/petroleum ether, m.p. 280-282°C (lit[40], 280-285°C) and a white crystalline solid (250mg) which was shown to be methyl indole-3-carboxylate.

Mixed melting point - 280-283°C (with authentic sample).

Elemental analysis - $C_{13}H_{12}N_4OS$; Requires - C,57.35: H,4.40: N,20.60.
Found - C,57.20: H,4.62: N, 20.74.

U.V. - λ_{max}(Methanol/ethanol)nm(ε); 364(5415), 278(9570), 272(9376) 215(25250).

I.R. - ν_{max}(Nujol)cm^{-1}; 3225(N-H), 1630(C=O).

lit[40] (KBr)cm^{-1}; 3225(N-H), 1630(C=O).

M.S. - (low eV, EI)m/z; 272(100%,[M^+]), 144(8%).

- (70 eV, EI)m/z; 272(56%), 144(100%), 129(14%), 102(28%).

N.M.R. - δ_H(d^6-DMSO)ppm; 12.2(1H,br.s,N-H), 9.05(1H,s,H-2), 8.36-8.14(1H,m,H-4), 7.68-7.56(1H,m,H-7), 7.36-7.24(2H,m,H-5, H-6), 3.25(6H,s,$N(CH_3)_2$).

- δ_C(d^6-DMSO)ppm; 187.8(s,C=O), 175.7+172.4(2s,thiadiazole C-3', C-5'), 138.3(d), 136.6(s,C-8), 126.3(s), 123.7(d), 122.8(d), 121.4(d), 112.8(d), 112.4(s), 38.6(t,$N(CH_3)_2$).

When the reaction was repeated using 2 molar equivalents of the oxathiazolone, the yield was slightly increased to 34%. Substitution of acetonitrile for methanol in the reverse phase solvent system, however, prevented formation of the methyl ester and allowed recovery of the unreacted acyl nitrile.

Attempted syntheses of 3-(N,N-dimethylamino)-5-(indole-3-methylene)-1,2,4-thiadiazole (63)

a) Cycloaddition of indole-3-acetonitrile (6) with 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68)

To a stirred solution/suspension of indole-3-acetonitrile (6) (25mg, 0.16mmol) in the relevant solvent (0.5cm^3) and heated to the required temperature was added 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68) (up to 100mg, 0.68mmol) at a rate of one drop per 20 seconds.

After addition, the solvent was removed and the product purified by column chromatography. The following solvents and temperatures were employed: chlorobenzene (130°C), xylene (140°C), DMF (140°C), diglyme (160°C), m-dichlorobenzene (180°C), methyl formamide (180°C), decalin (180°C), DMSO (180°C), p-nitrotoluene (180°C). In each case, however, the starting nitrile (6) was completely recovered from the reaction.

b) By the reduction of dendrodoine

i) Sodium borohydride:- To a stirred solution of dendrodoine (61) (50mg, 0.18mmol) in methanol ($1cm^3$) was added sodium borohydride (~50mg). As TLC analysis of the reaction mixture after one hour showed no change, it was heated to reflux. On heating, steady conversion of dendrodoine to a more polar compound began, a process which took one hour. As no further change was seen after a further hour at reflux, the solvent was removed and the resultant gum extracted between ethyl acetate ($5cm^3$) and water ($5cm^3$). The aqueous layer was washed with ethyl acetate ($5cm^3$), then discarded. The organic fractions were combined, washed with water ($2 \times 5cm^3$), dried ($MgSO_4$), filtered and evaporated, affording an orange gum (48mg), which was purified by column chromatography, to yield a colourless gum (~1-2mg), suspected from the mass spectrum to be the alcohol(84).

M.S. - (low eV, EI)m/z; 274(30%), 246(17%), 213(24%), 170(96%) 145(100%).

ii) Lithium aluminium hydride:- Dendrodoine (61) (50mg,0.18mmol) and lithium aluminium hydride (5mg, 0.7mmol) were stirred in dry THF ($5cm^3$) at -78°C for one hour. As no reaction was seen (TLC) to have

occurred after this time, the mixture was allowed to warm at 10°C intervals with one hour intervals in between. At -40°C slow converstion to the previous product began. On reaching room temperature, complete conversion had occurred and, as stirring for several more hours had no effect on the reaction mixture, saturated sodium potassium tartrate ($2cm^3$) was carefully added. The liquor was then decanted off and the residue washed with ethyl acetate ($2 \times 5cm^3$). The organic fractions were then combined, washed with water ($2 \times 10cm^3$), dried ($MgSO_4$), filtered and evaporated to afford an orange gum, which was purified by column chromatography to yield a colourless gum (1-2mg). TLC suggested this to be the same compound as previously attained.

iii) Diborane:- To dendrodoine (100mg, 0.37mmol) and sodium borohydride (~30mg, ~3 equiv) in dry distilled diglyme ($2cm^3$), stirred under nitrogen, was injected boron trifluoride etherate (150µl, 3 equiv). Five hours after the addition, TLC analysis still showed the presence of some starting material, so the reaction was left to stir for a further 12 hours (overnight). The solvent was then removed and the residue boiled with methanol ($10cm^3$) for 20 minutes. The methanol was then removed and the residue partitioned between ethyl acetate ($5cm^3$) and water ($5cm^3$). After washing with ethyl acetate ($2 \times 5cm^3$), the aqueous layer was discarded. The organic fractions were combined, washed with water ($2 \times 5cm^3$), dried ($MgSO_4$), filtered and evaporated to yield a yellow gum (95mg). TLC analysis of the gum showed two potential products, which were thought to be the alcohol and methylene compound. However, attempts to separate

them by chromatography yielded a mixture (8mg, 8%). Due to the exceptionally low yield, no attempts at further separation were made.

iv) Wolff-Kishner:- Dendrodoine (50mg, 0.18mmol), hydrazine (100µl, 3mmol) and potassium hydroxide (100mg, 2mmol), stirred in dry distilled digol ($2cm^3$) were heated for four hours at 170°C. The resultant reaction mixture was then poured onto crushed ice and extracted with ethyl acetate (2 x $10cm^3$). The organic layers were then combined, washed with water (2 x $20cm^3$), dried ($MgSO_4$), filtered and evaporated, to yield a yellow gum. TLC analysis of this gum showed all the starting material to have reacted to yield two less polar product spots, with very similar retention indices. The first attempt at chromatography separation of these two compounds failed, to yield a mixture (1-2mg) and because of the low yield of the reaction, no further separation was attempted.

v) Sodium cyanoborohydride:- To a solution of dendroine (50mg, 0.18mmol) in ethanol ($2cm^3$) was added concentrated sulphuric acid (2 drops) and sodium cyanoborohydride (~50mg, ~1mmol). The resultant mixture was stirred for a total of 72 hours over which time no reaction was seen to occur. On addition of water ($10cm^3$) to the reaction, a yellow precipitate formed (50mg) which was filtered, dried and shown by spectral analysis to be the starting material, dendrodoine.

c) By alkylation of 5-chloromethyl-3-(N,N-dimethylamino)-1,2,4-thiadiazole (86)

i) 5-Chloromethyl-3-(N,N-dimethylamino)-1,2,4-thiadiazole (86):-

To a solution of freshly distilled chloroacetonitrile ($2.5cm^3$, 40mmol) in decalin ($25cm^3$), stirred under nitrogen at 160°C, was added 5-(N,N-dimethylamino)-1,3,4-oxathiazol-2-one (68) ($0.5cm^3$, 4.5mmol) at a rate of one drop every 20 seconds. After the addition was completed, the reaction was stirred for a further five minutes before the solvent and excess chloroacetonitrile were removed under reduced pressure. The residue was then purified by column chromatography to yield an off-white solid (86) (400mg, 50%), crystallised from petroleum ether, m.p. 70-72°C.

M.S. - (low eV, EI)m/z;179,177(38%,100%,[M^+]), 102(8%), 44(22%).

- (70 eV, EI)m/z; 179,177(30%,78%,[M^+]), 102(100%), 87(41%), 44(99%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 4.80(2H,s,$C\underline{H}_2Cl$), 3.18(6H,s,$N(C\underline{H}_3)_2$).

- $\delta_C(CDCl_3)$ppm; 186.5, 172.1(2s,thiadiazole C-3,C-5), 40.2(t,$\underline{C}H_2Cl$), 38.9(1,$N(\underline{C}H_3)_2$).

ii) Attempted alkylation of the 5-chloromethyl-3-(N,N-dimethylamino)-1,2,4-thiadiazole (86):-

a) Reaction with indole magnesium bromide:-

To a stirred solution of the thiadiazole (86) (100mg, 0.56mmol) in dry THF ($5cm^3$) and under a nitrogen atmosphere was added indole magnesium bromide ($0.6cm^3$ of a 1M solution in THF, 0.6mmol). As

no reaction was seen to occur after 15 hours at room temperature, the mixture was heated at reflux for several hours. Neither this, nor the further addition of 3 molar equivalents of indole magnesium bromide with heating, however, could effect a reaction.

b) Reaction with 3-lithio-1-(phenylsulphonyl)indole (88):-
To 3-iodo-1-(phenylsulphonyl)indole[55] (100mg, 0.26mmol) in dry THF ($2cm^3$), stirred under nitrogen at -100°C, was added t-butyl-lithium (2 equivalents in dry THF) and the reaction stirred for five minutes before the further addition of a solution of the thiadiazole (86) (50mg, 0.28mmol) in dry THF ($0.5cm^3$). After this addition was completed, the reaction mixture was allowed to warm to room temperature over two hours before being poured onto crushed ice. The organic layer was then separated and the aqueous layer washed with ethyl acetate ($2cm^3$), then discarded. The organic fractions were then combined, washed with saturated sodium thiosulphate ($5cm^3$) and water ($2 \times 5cm^3$), dried ($MgSO_4$), filtered and evaporated, to afford a yellow gum (100mg). The gum was purified by column chromatography to yield a colourless solid (60mg), which was shown by spectral analysis to be 1-phenylsulphonylindole (91).

Attempted synthesis of 5-benzyloxydendrodoine (62)

5-Benzyloxyindole-3-carbonyl nitrile (92) (50mg) was heated over the same range of solvents used for the attempted cyclisation of indole-3-acetonitrile (6) and 5-(N,N-dimethylamino)-1,3,4-oxathiazole-

2-one (68). Up to five equivalents of the oxathiazolone were added in a portion-wise manner, and regular TLC analysis was taken. After the addition, and a further five minutes stirring, the solvent was removed and the residue purified by column chromatography. In each case no reaction was seen to occur, and all the starting material was recovered.

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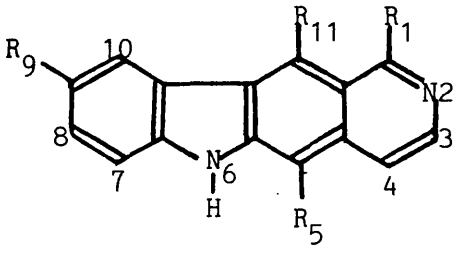
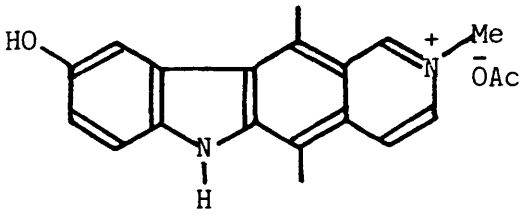
CHAPTER 3

A New Route to 6H-Pyrido[4,3-*b*]carbazoles

INTRODUCTION

A number of 6H-pyrido[4,3-b] carbazoles such as the 5,11-dimethyl derivative, ellipticine (94) the 1,5-dimethyl derivative, olivacine (95) and 9-methoxyellipticine (96) occur as alkaloids in plants of the Aspidosperma, Ochrosia, Tabernaemontana and Strychnos genera of the Apocyanaceae family[56]. They have all been shown to exhibit pronounced anti-neoplastic activity in several animal and human tumour systems and, in particular, against human myelobastic leukaemia[57,58].





(98)

(94) $R_5, R_{11}=CH_3$, $R_1, R_9=H$.

(95) $R_1, R_5=CH_3$, $R_9, R_{11}=H$.

(96) $R_5, R_{11}=CH_3$, $R_9=OCH_3$, $R_1=H$.

(97) $R_5, R_{11}=CH_3$, $R_9=OH$, $R_1=H$.

The synthesis of these compounds and their various substituted analogues has been the subject of continuous research for the last 25 years, this effort being largely stimulated by the desire to obtain new

derivatives for pharmacological evaluation. The most promising compound to date is 9-hydroxyellipticine (97), which, in the form of its metho salt[98], has proceeded through clinical trials to its use in France, for the treatment of human solid tumours. In Britain and the United States there remain doubts about the long term toxicity of these drugs, especially their mutagenic action, and so far no ellipticine analogue has received recognition as a major drug. This does not mean, of course, that efforts have ceased in these countries to find an acceptable compound in this series.

Ellipticine (94) and its analogues are known to be cytotoxic intercalating agents which act by entering the DNA double helix, thus impairing its effectiveness as a template for $_{m}$RNA transcription. It is also likely that ellipticines are not simply held in the helix by non-bonding forces. Recent work[59] suggests that these compounds are oxidised *in vivo* to iminoquinones which on entering the helix covalently bond to the DNA base pairs. This would explain the increased activity of the "pre-oxidised" 9-hydroxyellipticine over ellipticine itself, since part oxidation has already been achieved in the former structure. It also emphasizes the need for the development of a viable synthetic route to substituted ellipticines which may facilitate the binding process and/or their cell transport properties.

The synthesis of ellipticine and its derivatives

The syntheses of ellipticine and its analogues appearing in the literature between 1959 and the present day have been thoroughly reviewed in three articles, the first by Sainsbury[60] who reviewed studies from 1959 to 1976, the second by Hewlins *et al*[61] and just

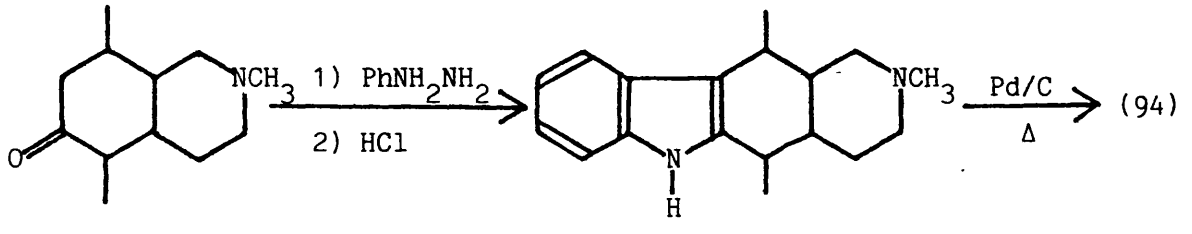
recently by Gribble and Saulnier[62] who brought the previous review up-to-date.

To go over this topic in full once again is obviously superfluous, and the author will therefore only pick out those syntheses which highlight the various approaches to the tetracyclic system.

In the first of the reviews, Sainsbury[60] categorises the syntheses according to which ring of the tetracycle is formed last, B, C or D, and this is also the system to be used here. It should be noted that to date no synthesis has been reported where the A ring is created in the final step.

B type syntheses

This bond forming strategy was first employed by Woodward and Stillwell[63] who used a Fischer indolisation reaction to synthesise an octahydroellipticine (100) in 82% yield from the decahydroisoquinolin-6-one (99). N-Demethylation was effected by hydrogenolysis over palladium on charcoal and oxidation to ellipticine was similarly achieved by dehydrogenation over the same catalyst (Scheme 34).



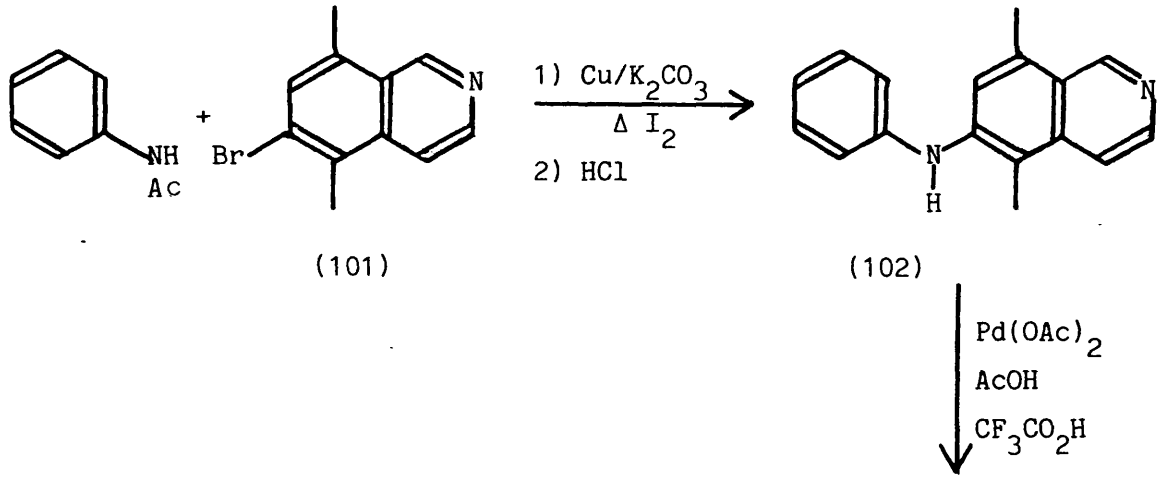
(99) (100)

SCHEME 34

Although the initial reaction was very efficient, the final N-demethylation/oxidation process only produced ellipticine in 0.3% yield.

Recently, however, a French team[64] used this method to synthesise a series of 9-substituted 11-demethylellipticines, claiming yields for the final aromatisation process of ~26%. The overall process is still, however, unpromising because of the relative inaccessibility of the starting decahydroisoquinolinones (99).

An alternative B-type synthesis was recently reported by Miller and Moock[65] in which the bromoisoquinoline (101) was coupled, using a Goldberg modification of the Ullmann reaction, with acetanilide to give, after hydrolysis, the diarylamine (102). The final ring closure to ellipticine was effected by heating with palladium acetate (Scheme 35).



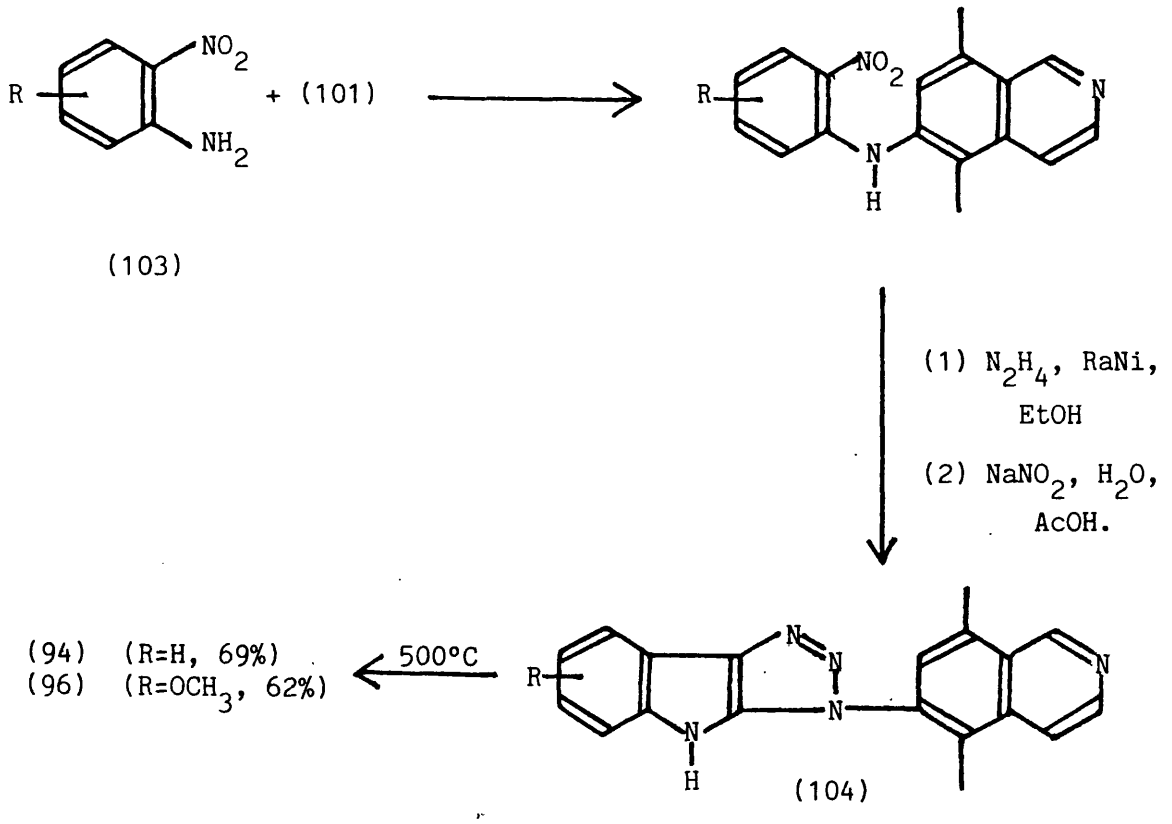
(101) (102) (94)

SCHEME 35

As the yield for this final reaction was again low (15-25%), Miller[66] later changed his synthesis, effecting the coupling reaction between o-nitroanilines (103) and the bromoisoquinoline (101). Should this be an ionic reaction, then the electron-withdrawing nitro group would have an

inhibitory effect. The fact that it has the opposite effect indicates strongly that the reaction is radical in nature, although the precise role of the copper is still unclear.

The nitro group of the coupled products was reduced and diazotized to yield the corresponding benzotriazoles (104) in over 90% yield. Pyrolysis of this compound gave the required ellipticines (94) and (96) in good overall yields (Scheme 36).



SCHEME 36

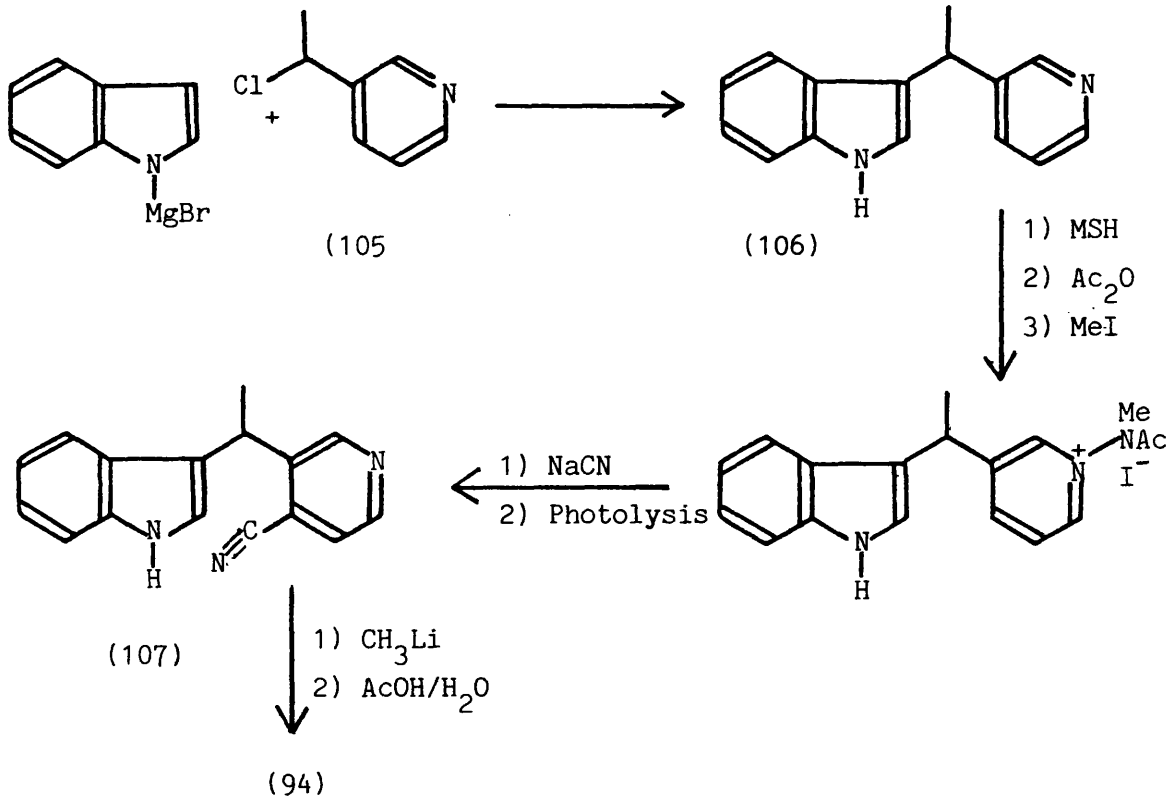
The major criticisms of this synthesis are (a) the relative difficulties in obtaining large supplies of the bromoisoquinoline (101) and (b) the harsh conditions of the final pyrolysis step: no one can

contemplate with equanimity the prospect of exposing a hard won intermediate to a temperature of 500°C!

C Type syntheses

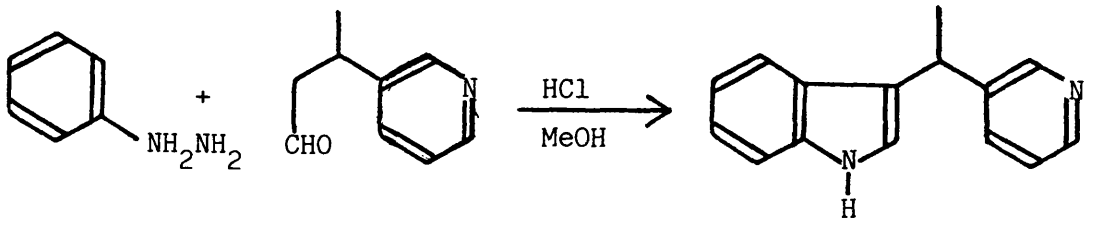
There are three basic strategies used in the construction of the C ring as the ultimate step.

The first is typified by Sainsbury's[67] approach to ellipticine, in which indole magnesium bromide is reacted with 3-(1-chloroethyl)-pyridine (105) to yield the 1:1 adduct. Functionalization of the pyridine nitrogen of this adduct(106) with O-mesitylene sulphonyl hydroxylamine (MSH), acetic anhydride and methyl iodide facilitates the already electron deficient pyridine ring to regioselective nucleophilic attack by sodium cyanide. This gives a dihydropyridine intermediate which degrades on photolysis to yield the nitrile(107). Treatment of the nitrile(107) with methyllithium and subsequent mild hydrolysis, with dilute acetic acid, afforded ellipticine (94). Overall this is a very useful approach and all the steps from the indolyl methanes(106) work well; unfortunately, the yield in the reaction between indolyl Grignard reagents and 3-(α-halo)ethyl-pyridine is inefficient (Scheme 37).



SCHEME 37

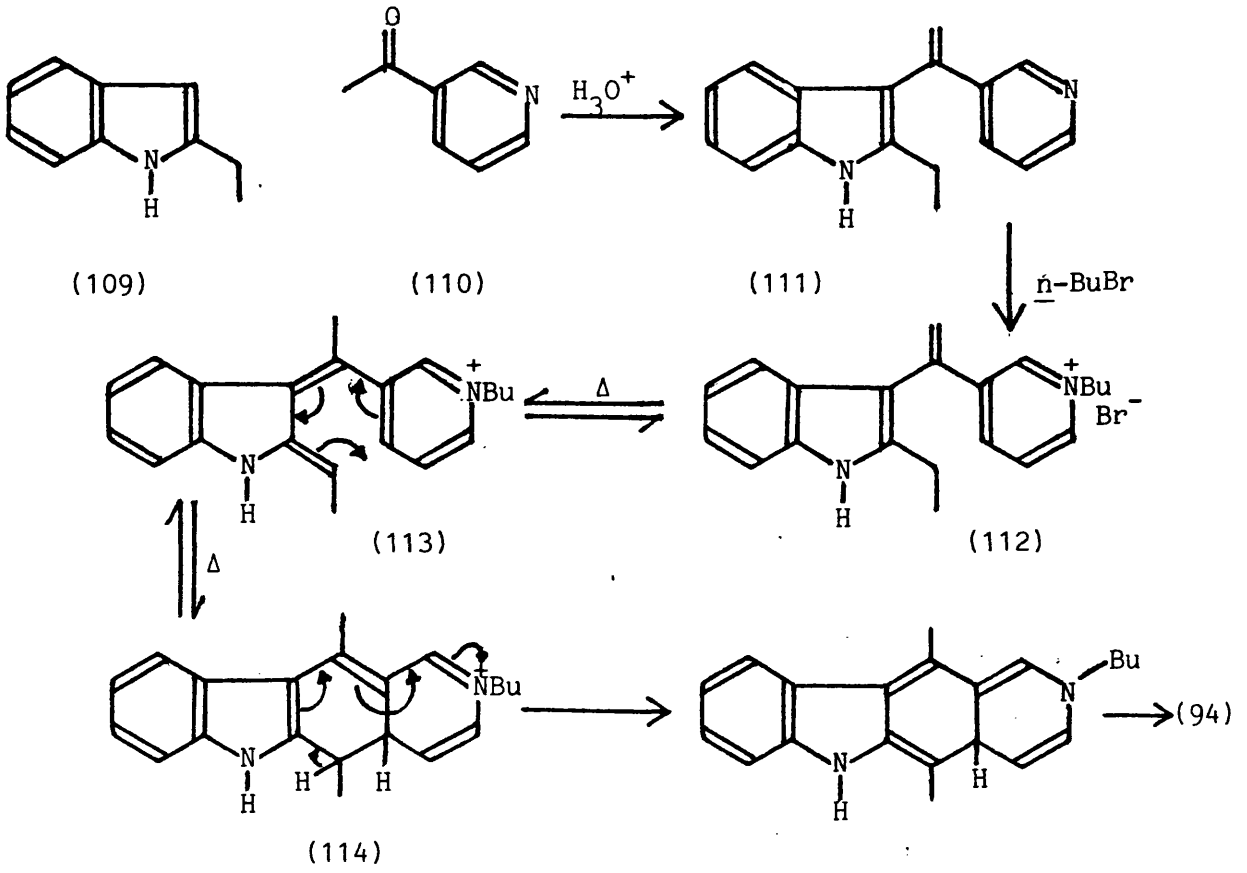
However, Sainsbury and Weerasinghe[68] reported an improved synthesis of the key intermediate(106) using a Fischer indolisation reaction upon the accessible aldehyde (108) (Scheme 38).



SCHEME 38

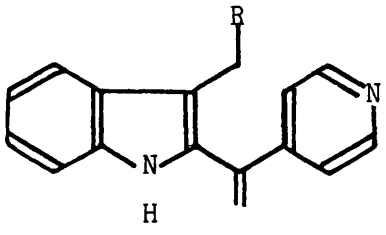
In another version of this type of ring construction, the cyclisation of the C ring is effected by the thermal generation of a hexatriene through its disrotatory ring closure. This, of course, gives a cyclohexadiene intermediate which may be dehydrogenated to the aromatic species at the end of the synthetic sequence.

A classic example of this approach was reported by Bergmann and Carlsson[69] in 1977. Here the vinylindole(111) is prepared by the condensation of 2-ethylindole(109) and 3-acetylpyridine (110) and then quaternised by treatment with butyl bromide. This product (112) is then pyrolysed and presumably undergoes thermal isomerisation to the triene (113) prior to cyclisation to the intermediate (114), which under the severe conditions employed ~ 500° - 600°C loses hydrogen bromide and butane, perhaps through an intramolecular mechanism to afford ellipticine (94) in 72% yield (Scheme 39).

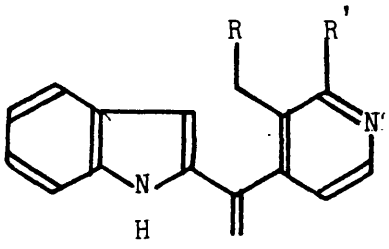


SCHEME 39

Kano et al have also used the same concept to prepare 6H-pyrido-[4,3-b] carbazoles from compounds of type (115)[70] and (116)[71].

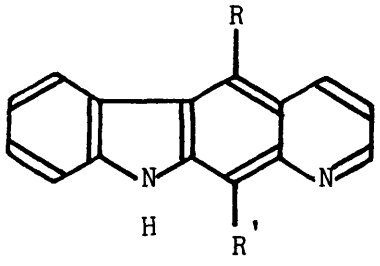


(115)



(116)

These reactions work reasonably well, but once again, however, high temperatures are necessary to effect cyclisation and, as in the Bergmann/ Carlsson synthesis, isomeric structures can result, as there are two available sites on the pyridine ring in the substrate(111)at which bond formation may occur, allowing the possible formation of the "isoellipticine" (117).

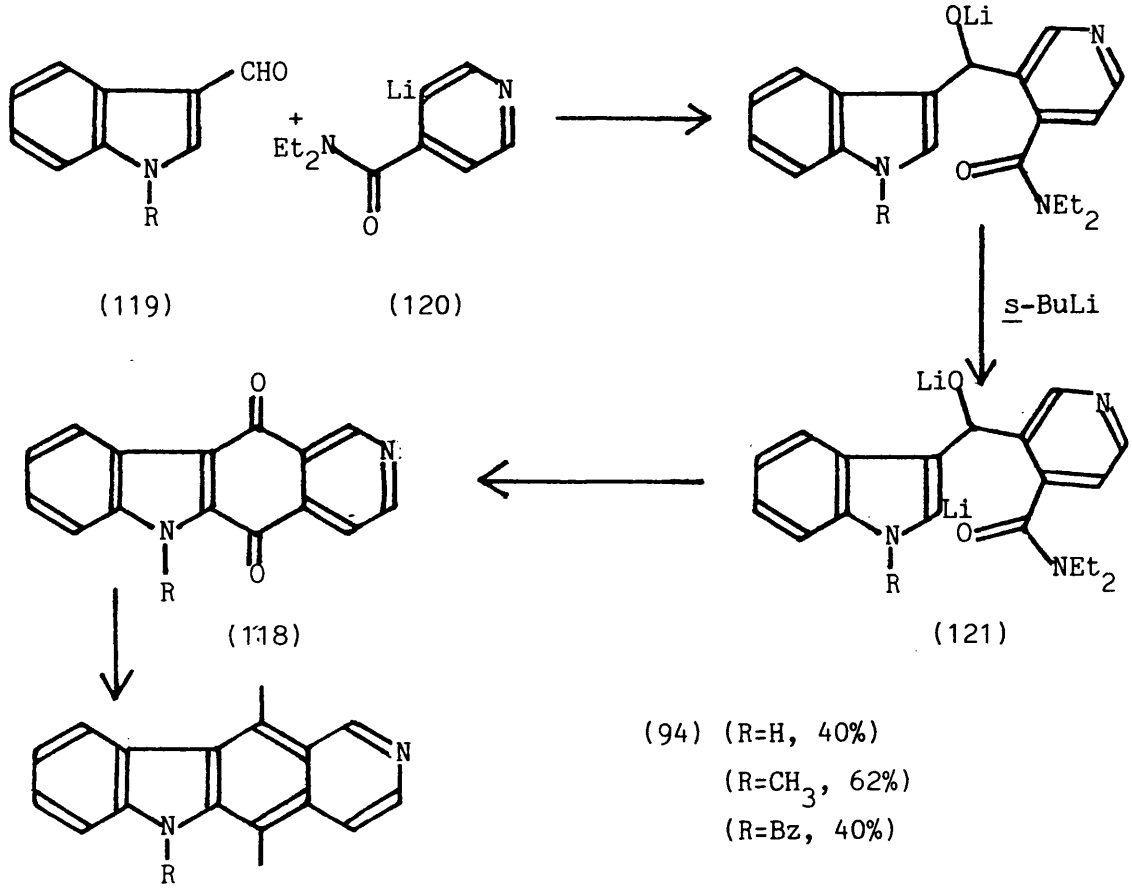


(117)

A final illustration of this class is the synthesis of quinone intermediates, the subsequent reductive alkylation of which leads to 6H-pyrido[4,3-b] carbazoles.

An excellent example of this type of approach is reported by Snieckus and Watanabe[72] in which the required quinone(118) was prepared by the reaction of a N-protected 3-formylindole (119) with lithiated N,N-dimethylisonicotinamide (120)in the presence of excess sec-butyllithium.

The reaction is presumed to take place via an intermediate lithiated species (121). The quinone (118) is converted to the corresponding ellipticine by treatment, first with methyllithium, and then with hydrogen iodide, stannous chloride and hydrochloric acid (Scheme 40).



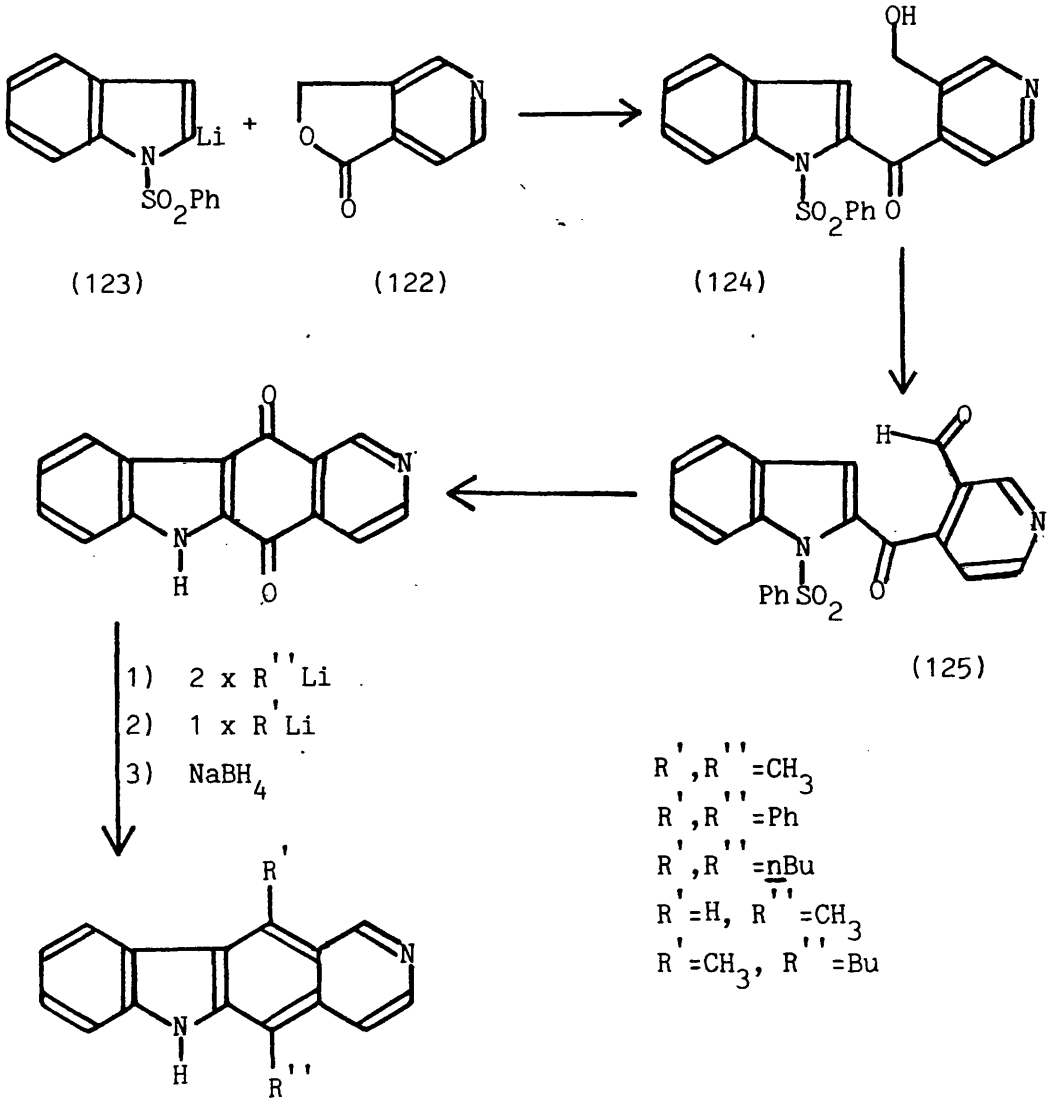
(119) (120) (121)

(118)

(94) (R=H, 40%)
(R=CH_3, 62%)
(R=Bz, 40%)

SCHEME 40

The quinone(118) (R=H) has also been prepared by Joule[73], who has shown that the pyridolactone (122) reacts with 2-lithio-1-benzenesulphonyl-indole (123) to afford the ketone (124). This was then oxidised to afford the ketoaldehyde(125), which as the acetal may be N-deprotected and then cyclised to the quinone by treatment with hydrochloric acid in the presence of air (Scheme 41).



SCHEME 41

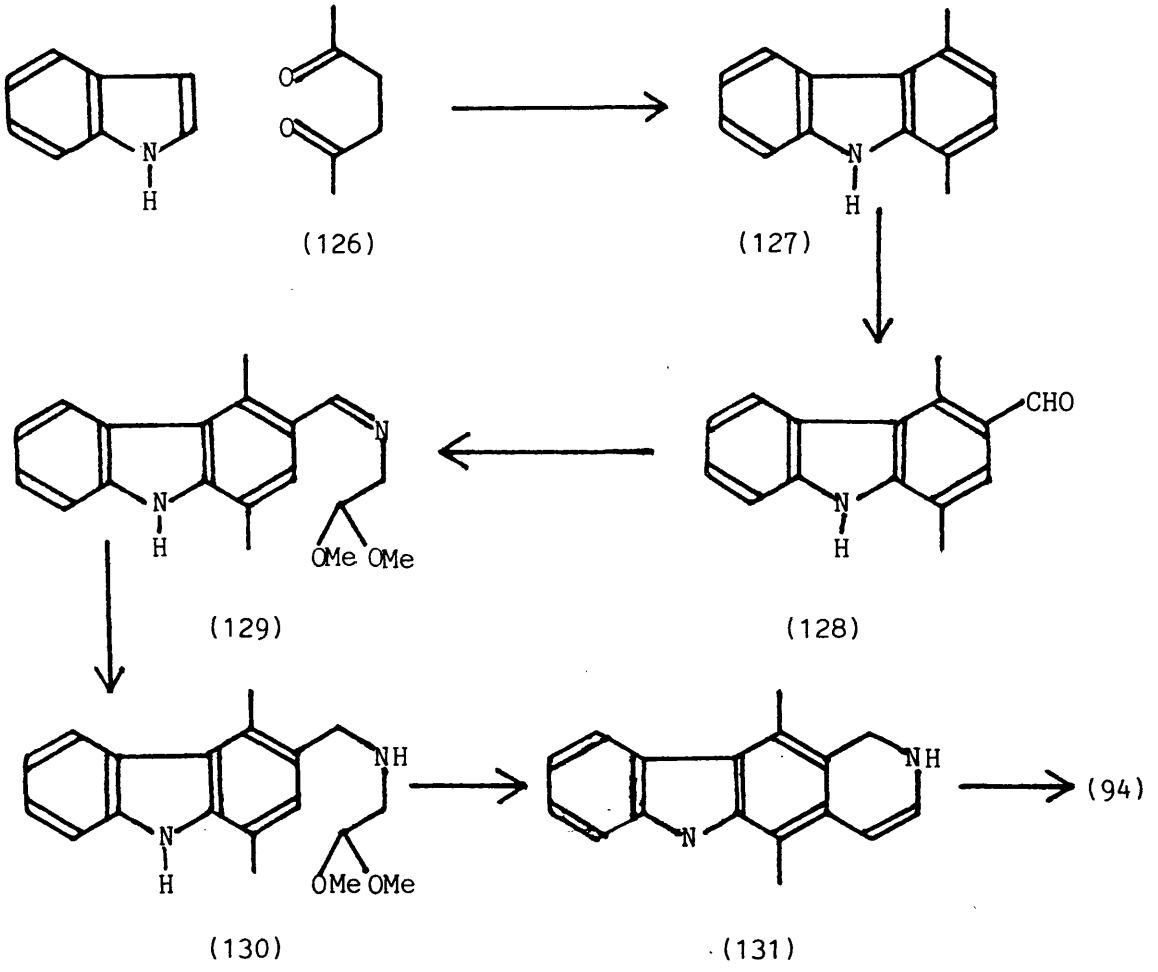
In a development of this route Joule then showed that if the quinone(118) is treated with two different alkylating agents in sequence, the more reactive 5-position of the quinone is alkylated first, followed by attack at the 11-position, thus allowing the synthesis of ellipticine with varying 5- and 11-substituents.

The only draw-back to this method is the relative inaccessibility of the pyridolactone(122) which is prepared in three steps from dimethyl pyridine-3,4-dicarboxylate in 16% overall yield.

D type syntheses

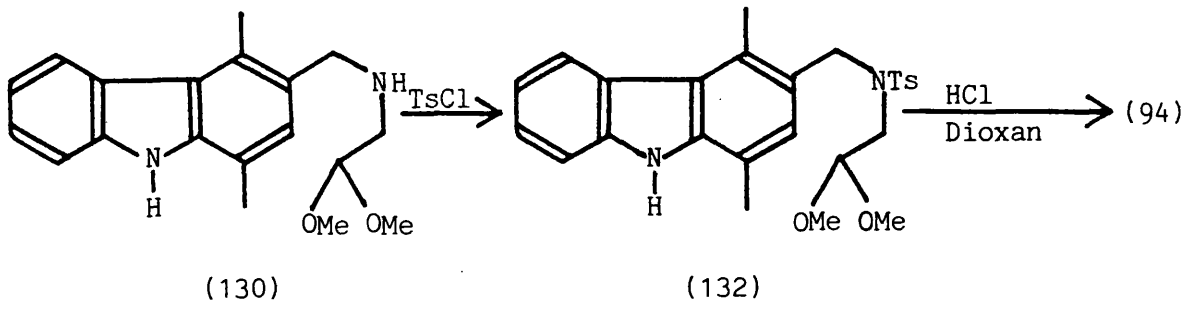
In this approach rings A, B and C, performed as a carbazole unit, are alkylated either at C-2 or at C-3, and D ring is then constructed by standard methods.

Probably the most commonly used synthesis in this class was originally devised by Cranwell and Saxton[74]. In this route indole is condensed with 2,5-hexanedione(126) to give 1,4-dimethylcarbazole (127). Subsequent formylation of this carbazole(127) gives the 3-formyl derivative (128) which is condensed with aminoacetaldehyde dimethyl acetal to yield the imine(129). Reduction of this imine(129) to the amine(130) and acid treatment effects cyclodehydration to the dihydroellipticine (131). This is subsequently oxidised to ellipticine (94) by heating with palladium on charcoal (Scheme 42).



SCHEME 42

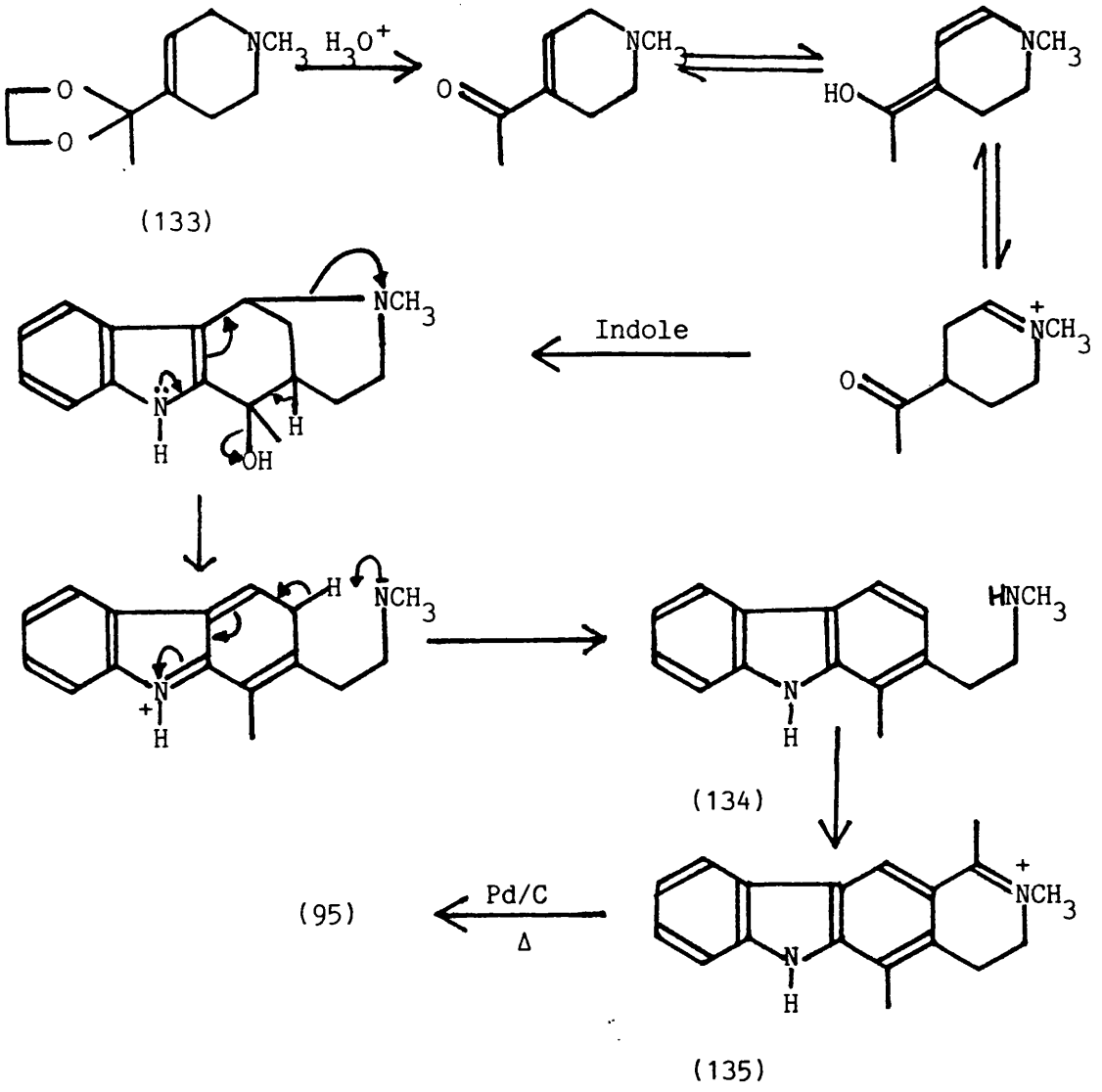
Jackson et al[75] have greatly improved the yield of the cyclisation reaction by first converting the amine(130) to the tosyl derivative(132) Treatment of this derivative(132) with hydrogen chloride in dioxan then gave ellipticine, since cyclodehydration and loss of p-toluene-sulphinic acid occur concurrently, thereby avoiding the difficult thermally promoted oxidation step (Scheme 43).



(130) (132)

SCHEME 43

The D ring can also be generated from 2-substituted carbazoles arranging subsequent ring closure onto the 3-position. A novel and elegant synthesis of olivacine (95) by Besselièvre and Husson[76] serves as a good example of this approach. Indole was condensed with the dihydropiperidine acetal (133) to give the aminocarbazole (134) in a single operation (74% yield). This somewhat unexpected product is formed under acidic conditions through the isomerization of the acetal prior to condensation with indole, followed by cyclisation/decyclisation as indicated in Scheme 44. The carbazole was then converted to olivacine in a conventional way, ie N-acetylation, followed by a Bischler Naperialski cyclisation to the 3,4-dihydroisoquinolinium salt (135), dehydration and N-demethylation.

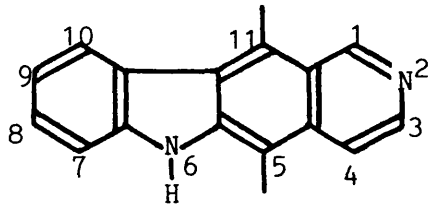


SCHEME 44

In conclusion, it must be said that despite the volume of work carried out and the number of routes available, there is still not one generally applicable synthesis of ellipticine and its derivatives.

DISCUSSION

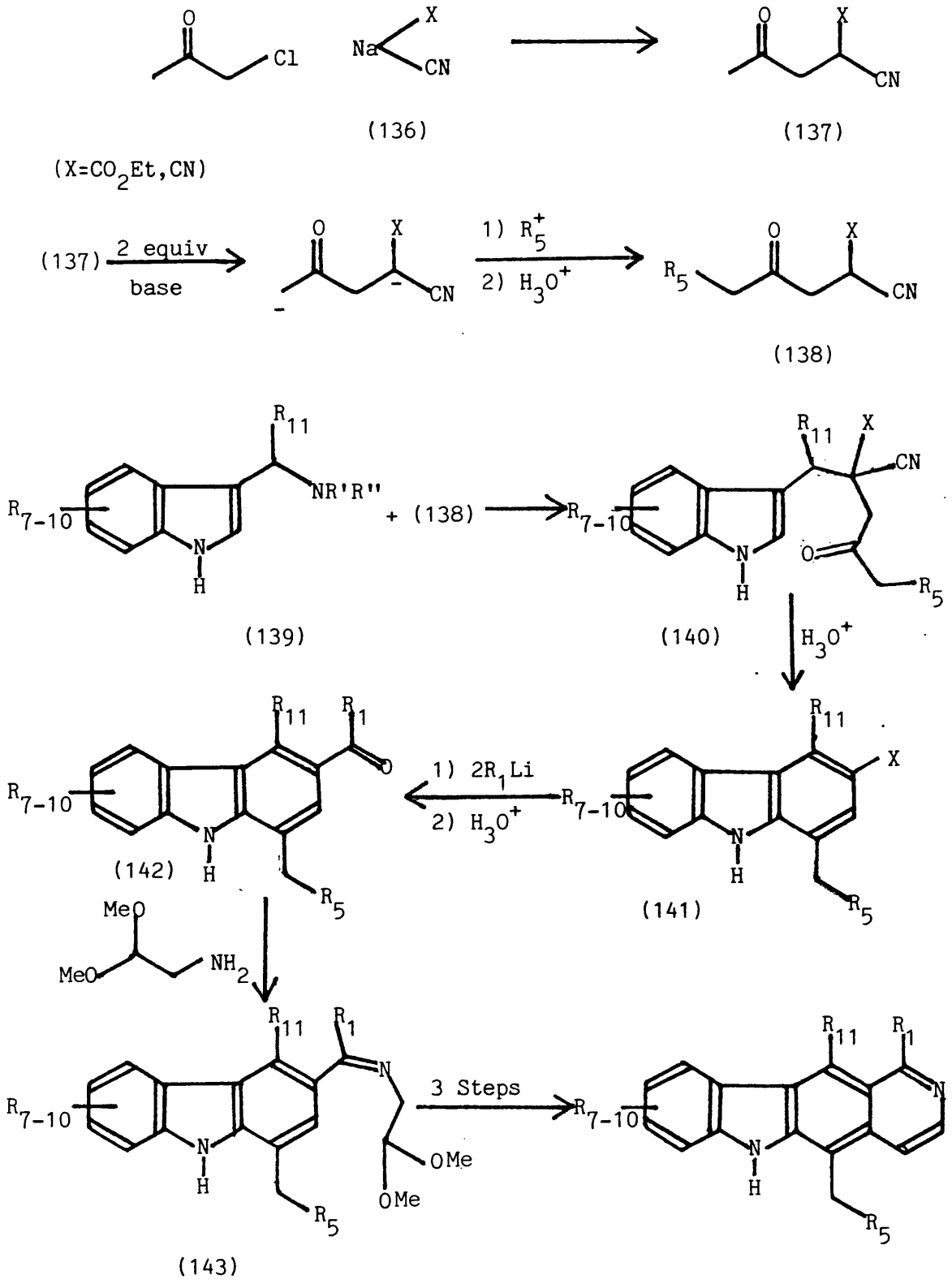
An ideal synthesis of ellipticine (94), and its derivatives, would be one which uses cheap, readily available starting materials, can be carried out on any scale and allows substitution at various positions of the tetracycle. It was with these criteria in mind that we approached the synthesis of ellipticine (94).



(94)

Ellipticine

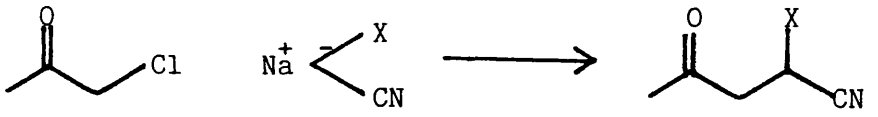
Our proposed route is outlined in Scheme 45.



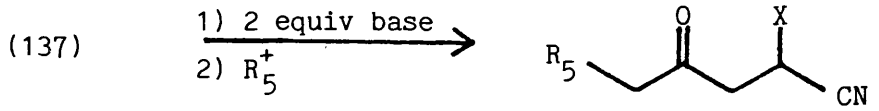
SCHEME 45

The problems posed, or the advantages presented, by each of the steps in this sequence are outlined below.

The first step involves the synthesis of a 2-substituted 4-oxopentanonitrile (137) and, if necessary, its terminal alkylation. (Scheme 46).



(137)



(138)

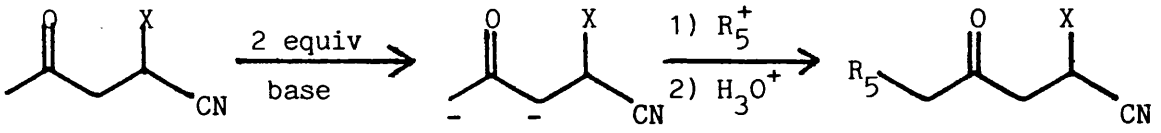
(a) $X=CO_2Et$

(b) X=CN

SCHEME 46

Ethyl 2-cyano-4-oxopentanoate (137a) has been successfully synthesised by the route[77] shown above using sodium ethoxide as the base and ethanol as the solvent. Although a synthesis of 2-cyano-4-oxopentanonitrile (137b) has not been previously reported, there seems no apparent reason why a similar condensation reaction between malononitrile and chloroacetone should not yield the required product (137b).

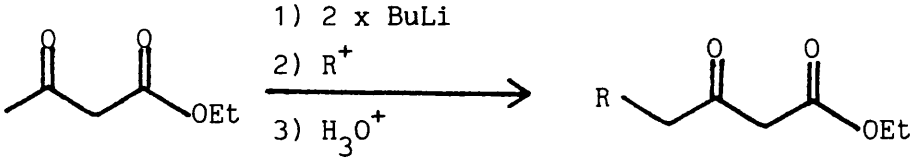
Our next requirement is the selective alkylation of either (137a) or (137b) at the methyl group of the acetyl moiety. This involves the generation of the dianion of (137) with two equivalents of strong base. We reasoned that the first equivalent will remove the most acidic proton forming an anion at the methine carbon. The second, due to the inherent instability of an α, β dianion will remove a proton from the methyl group, thus forming an anion at the terminal carbon. Under kinetic control, alkylation of the dianion should occur at the reactive terminal carbon, thus presenting easy access to a wide variety of substrates of the desired type. (Scheme 47).



(137) (138)

SCHEME 47

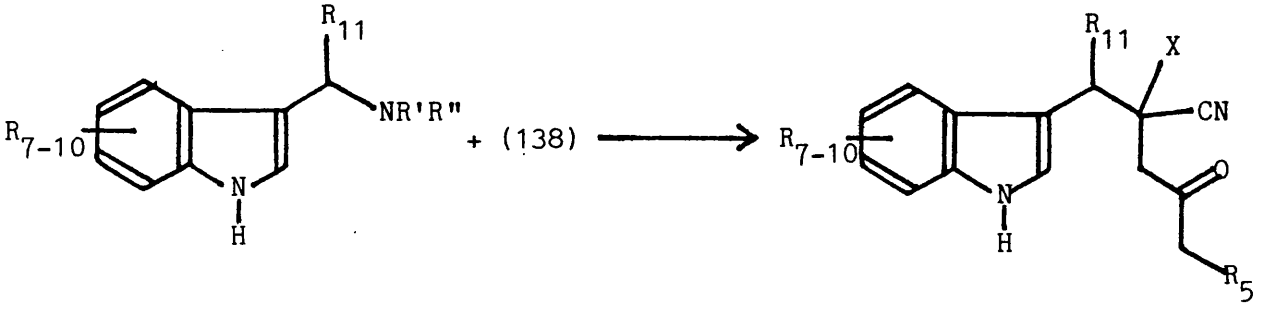
There is certainly precedence in literature to support this conclusion. Work carried out on dianions of β-diketones[78] and β-ketoesters[78,79] shows that they give only terminal alkylation products in high yields when reacted in this way. (Scheme 48)



SCHEME 48

Although alkylation of the terminal carbon is unnecessary in the synthesis of both ellipticine and olivacine, where R_5=H, it is important for the preparation of other ellipticine derivatives.

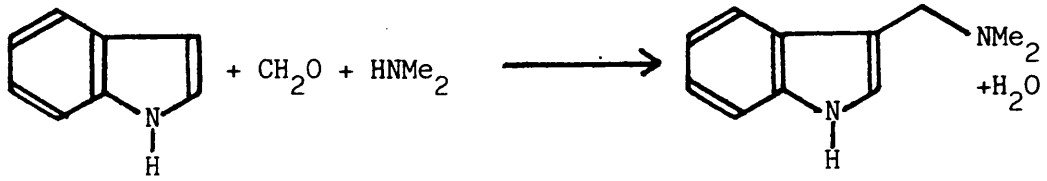
Step two requires the combination of the indolylic unit and the alkylated cyanoketone. As illustrated, a gramine-like base is to be employed and the choice of these compounds is obvious from the following commentary. (Scheme 49).



(139) (140)

SCHEME 49

Gramine(139a)(R_{7-10},R_{11}=H;R',R''=Me) is a Mannich base, so named because it is synthesised by the Mannich reaction[80].

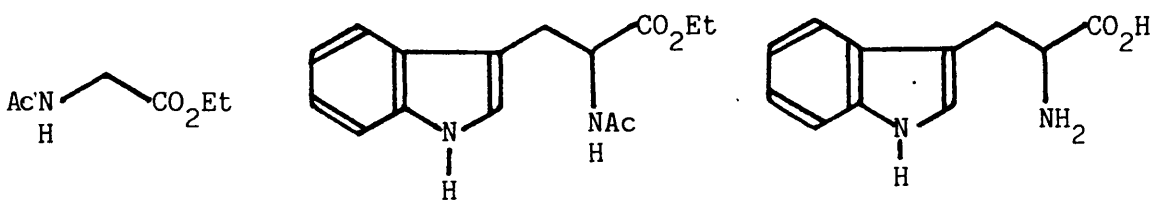


(139a)

SCHEME 50

Gramine(139a) and its quaternary salts have been used for many years to introduce an indole-3-methylene moiety into compounds containing an active methylene group.

Most of the early work (circa 1945) was carried out by H R Snyder and his various associates[81,83,85,86,87,121]. They were particularly interested in the coupling of gramine(139a) with ethyl 2-acetaminoacetate (144) to yield ethyl 2-acetamino-3-(indol-3-yl)-propanoate(145) a precursor to tryptophan(146).



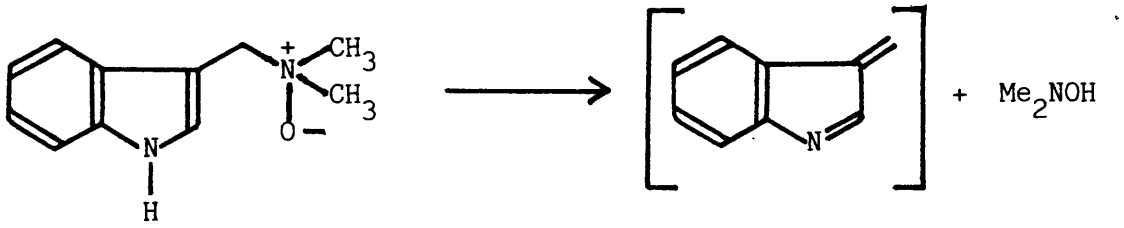
(144) (145) (146)

although this is the most likely route, 1-methylgramine, which cannot undergo amine elimination, is still an alkylating agent [81,83,86]. This would indicate that elimination is not the only possible mechanism, and direct substitution by either an S_N1 or S_N2 process must also be considered.

The three most common quaternary salts used are the methiodide [87], ethiodide [92,93], and the methiosulphate [94]. These are normally generated in situ by the addition of methyl iodide, ethyl iodide and dimethyl sulphate respectively to gramine in a polar solvent, usually absolute ethanol. These gramine quaternary salts are then reacted with the sodium salt of the relevant activated methylene compound.

Although originally the quaternary salts were prepared before the addition of the sodio methylene compound, enhanced yields have been obtained [92,95], when the quaternising agent was added dropwise to a mixture of gramine and the sodio methylene compound.

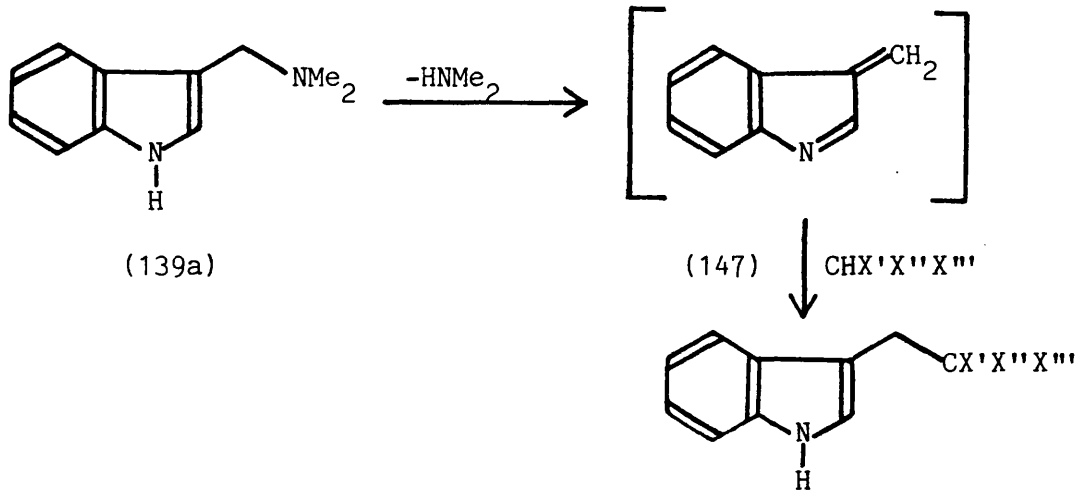
Gramine oxide(148) has also been used [96], as an alkylating agent, as it too can eliminate to give the active olefinic species (147). However, yields are poor.



(148) (147)

SCHEME 52

The mechanism most frequently proposed[81] for this reaction involves the elimination of dimethylamine to generate a methylene indolenine intermediate(147) which is then trapped by the latent nucleophile compound.



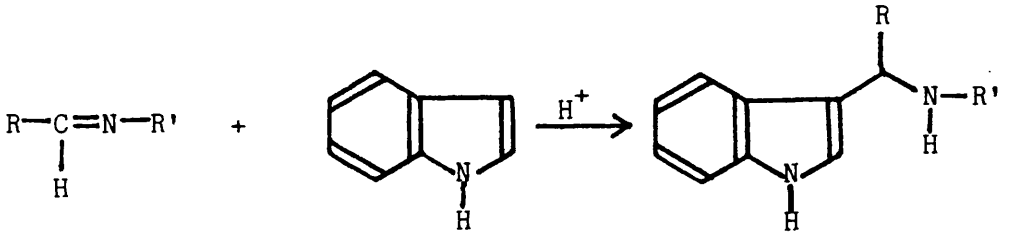
SCHEME 51

The reaction is often initiated by a catalytic amount of strong base usually sodium hydroxide[82], although this is not always necessary[83,84]. Inert aromatic hydrocarbons, such as toluene or xylene, are normally used as soluents heated to reflux temperatures. However, an excess of the active methylene compound has been used as solvent. This method is particularly useful with nitroalkanes[85], where diindolisation of the active methylene compound can present a problem.

More recently other catalysts have been employed[88,89], including tertiary phosphines[90,91], which are only mildly basic and have been used particularly where dialkylation may occur.

It has been suggested that the alkylation of gramine quaternary salts occurs by the same elimination-addition mechanism. However,

Attempts to use aldehydes other than formaldehyde to form Mannich bases with indole and dimethylamine have met with limited success, partly due to the reduced activity of the carbonyl species. However, ∝-substituted indole-3-methylamines of the Mannich type, "pseudo"-Mannich bases, have been obtained by the addition of indoles to aldimines(149) in the presence of an acid catalyst, usually glacial acetic acid. (Scheme 53).



(149)

SCHEME 53

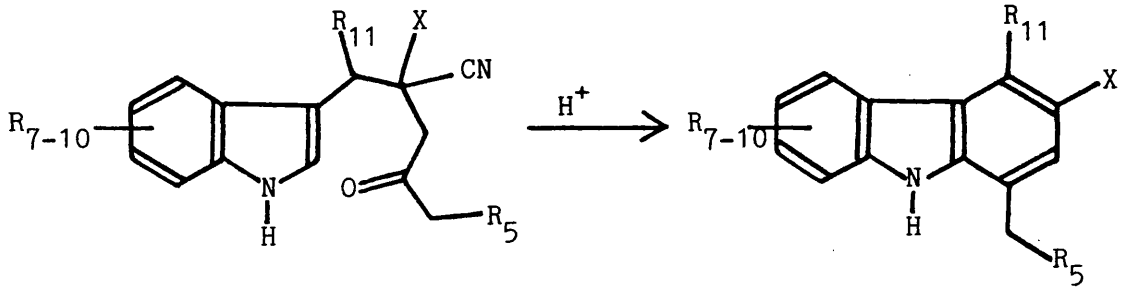
Aldimines are simply prepared[98] by condensation of the relevant aldehyde with the appropriate primary amine. Snyder and Matteson[99] have successfully used one of these indole "pseudo" Mannich bases where R=Me and R'=isopropyl, to alkylate acetaminoacetate esters (144), under the same conditions as used for gramine(139a) with yield around 90%.

A large number of these "pseudo" indole Mannich bases have been synthesised by the previously described method[98,99,100,102,103], thus, in principle, allowing the substituent on the 11-position of the ellipticine tetracycle to be varied as required during the implementation of our approach.

In the case where $R_{11}=CH_3$, the relevant gramine derivative has already been synthesised, but by a non-Mannich route[104], and has been used successfully as an alkylating agent in the synthesis of ellipticine by Le Goffic and Gouyette[104].

Another advantage of the projected route is that it enables the prior functionalisation of the indole moiety, for apart from strongly electron withdrawing groups in the carbocycle, such substituents are unlikely to interfere with the alkylation process. Already a variety of such substituted gramines are known[102,103]. Of particular interest are 5-alkoxylated gramines, since they might ultimately lead to 9-hydroxylated ellipticines with potential anticancer activity. Indeed, 5-methoxygramine has been used by Gouyette et al[105] in their synthesis of 9-methoxy-11-desmethylellipticine.

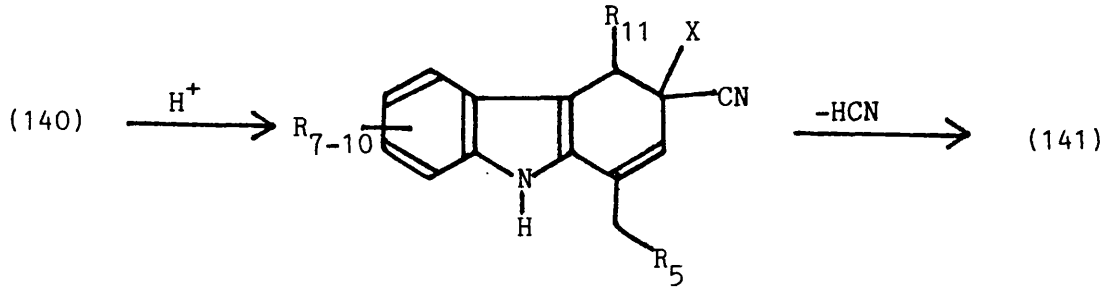
Having secured the general precursor (140) the next problem is the formation of the carbazole (141). This constitutes step three, and presents a question of orientation.



(140) (141)

SCHEME 54

As specified, the alkylation product(140) is required to undergo an acid catalysed ring closure to the 2-position of the indole moiety followed by dehydration to the 3,4-dihydrocarbazole (150). This may then eliminate hydrogen cyanide to afford the carbazole (141).

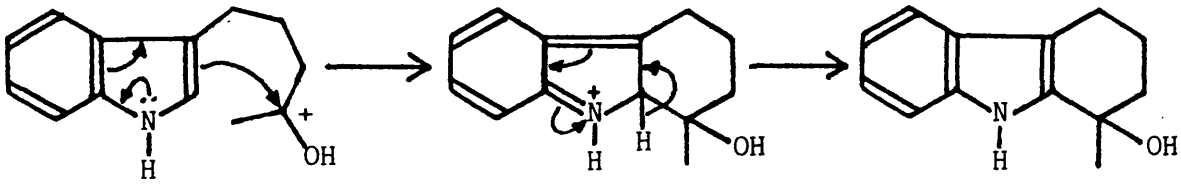


(150)

SCHEME 55

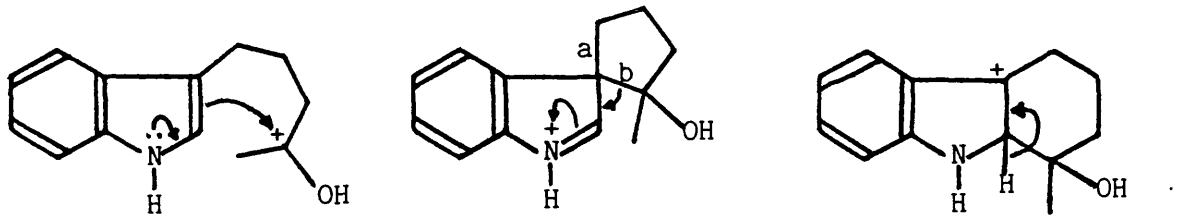
Similar ring closures have often been used in the synthesis of ellipticine and its analogues. The acid catalyst is usually acetic acid [106,107,108], although boron trifluoride etherate[109] and p-toluene sulphonic acid[109] have also been employed.

There are two possible mechanisms for this ring closure. The simplest involves direct attack of the protonated ketone onto the 2-position of the indole, followed by loss of the 2-indole proton.



SCHEME 56

The second course often argued by Jackson and Smith[110] suggests that the initial substitution occurs at the more reactive 3-position of the indole ring to give a 3,3'-spirocyclic indolenine(151). This then follows a Wagner-Meerwein[111] type 1→2 rearrangement to the cation(152) which deprotonates to the tricycle. (Scheme 57)

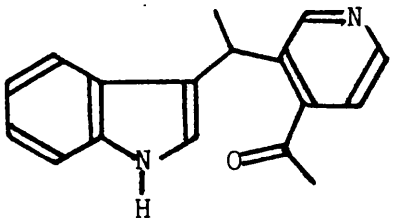


(151) (152)

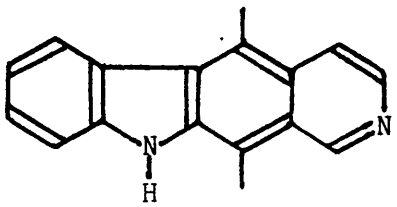
SCHEME 57

In substituted precursors such as ours, a problem could arise if the wrong bond migrates in the spiro intermediate, ie bond (a) instead of bond (b), as the resultant carbazole would have the substituents on the 1,2 and 3,4 positions inverted. Migration of the correct bond (b) should be facilitated by electron release from the oxygen according to Jackson and Smith[110]. Sainsbury et al[112] have studied this problem with the ring closure of ketone (153).

They have shown on the basis of spectral evidence, that ellipticine, and not isoellipticine (154) was obtained as the sole isolated product.



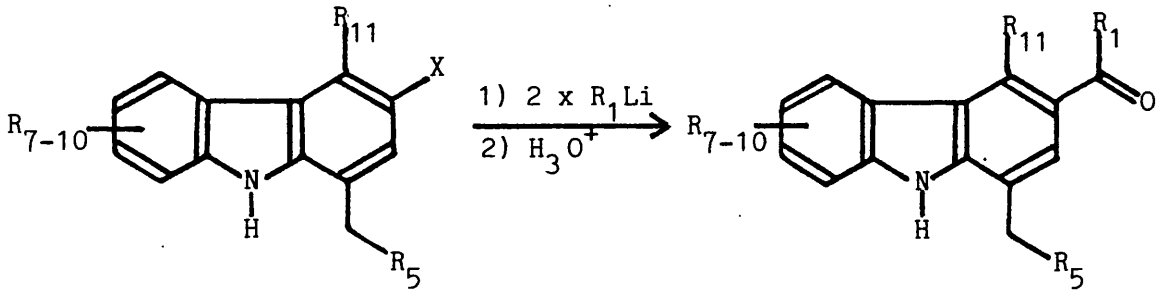
(153)



(154)

Also in cases[106,109,112] where the migratory aptitude of the two bonds is not so apparent, structure integrity is maintained.

If, in our case, the desired carbazole (141) is produced, the fourth step is its conversion into the acyl derivative (142). (Scheme 58)

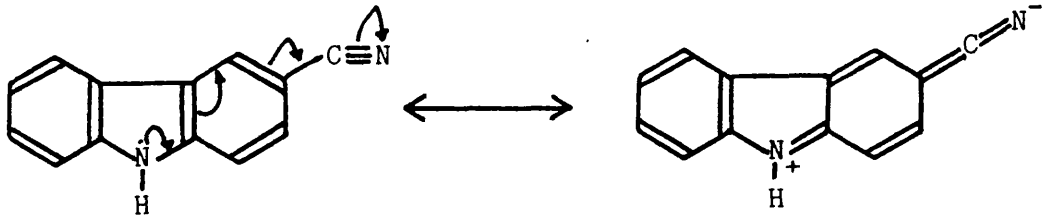


(141) (142)

SCHEME 58

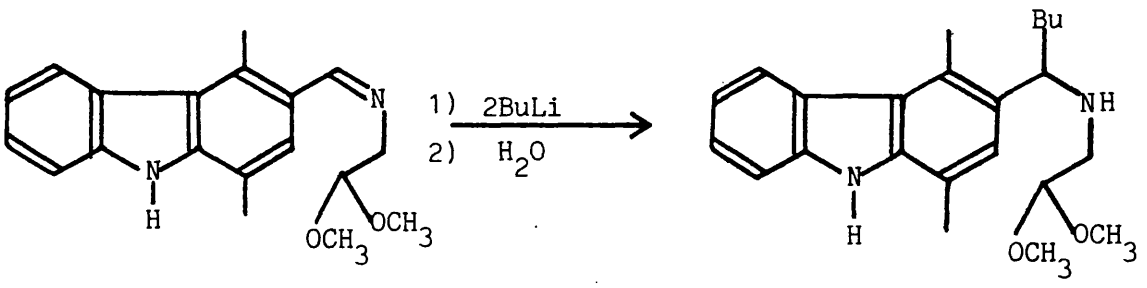
In this process an alkyl lithium is likely to be the reagent of choice, but two molecular equivalents will be required as the first would simply deprotonate the acidic carbazole NH group.

Problems could also arise because of the deactivating mesomeric effect of the carbazole nitrogen's lone pair on the <u>para</u> orientated nitrile function. Indeed this effect would be enhanced by deprotonation.



A novel way to solve this problem, if it arises, would be to N-acetylate the carbazole, thus deactivating the nitrogen, and to treat this with two separate equivalents of alkyl lithium. Assuming the nitrile is the more reactive centre, the first equivalent of alkyl lithium should alkylate it and the second cleave the protecting group. If this fails, an N-sulphonyl protecting group could be used.

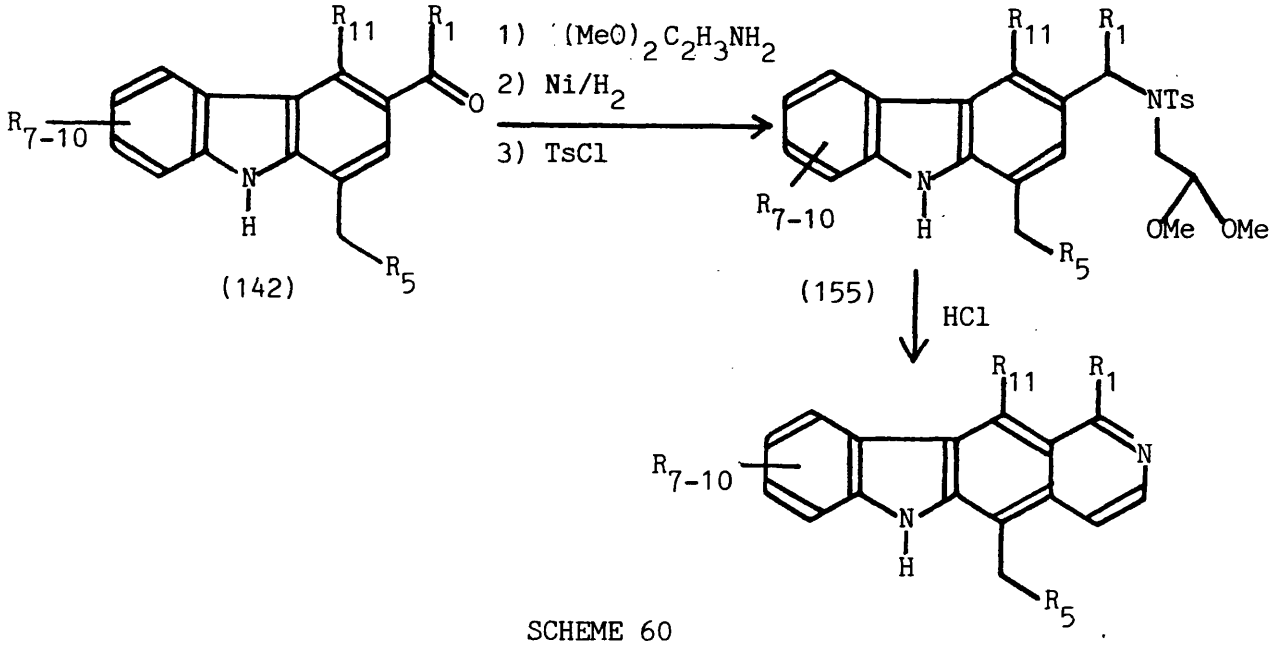
Sainsbury and Smith[113] have successfully alkylated imines of the type (143), where R_1=H , by treatment with two molecular equivalents of n-butyl lithium. An example is given below:



SCHEME 59

Therefore, if the proposed alkylation of the carbazole (141) were to fail, the nitrile (or ester) could easily be converted through the aldehyde to the imine and this product then be alkylated, as in the cited example.

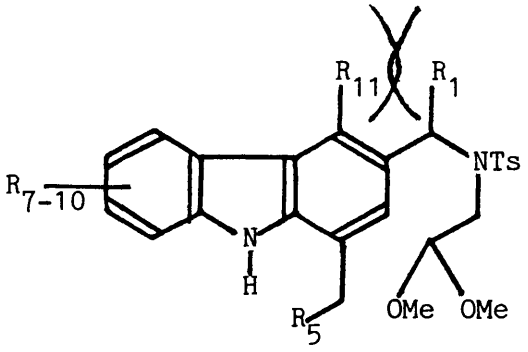
At the stage of the acylated carbazole, few further problems were were envisaged, in the general case, as there are ample illustrations in the literature for the completion of the overall synthesis.



SCHEME 60

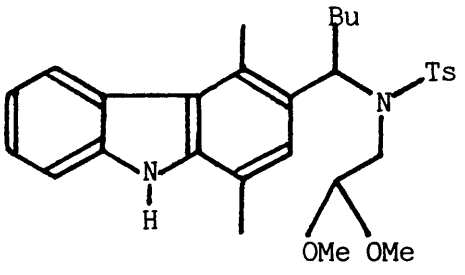
Indeed, this is a standard ring closure in the syntheses of ellipticine[115], 11-dimethylellipticine[116] and olivacine[116], as devised by Cranwell and Saxton[114] and subsequently modified by Birch, Jackson and Shannon[115].

If, however, both R_1 and R_{11} are substituted, then problems may arise, as peri interactions between the two groups may prevent the 'tosyl' amine(155)from obtaining the required conformation for cyclisation.



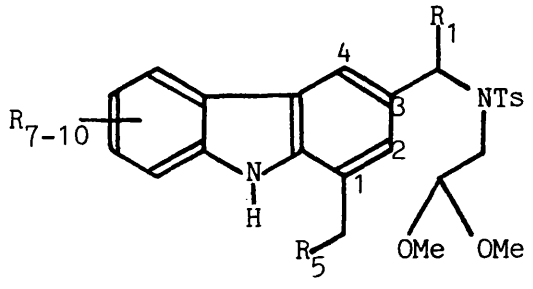
(155)

For example, Sainsbury and Smith[113] recently attempted to prepare 1-butylellipticine R_{11} = Me, R_1 =Bu and failed to effect the final cyclisation of the intermediate (155a).



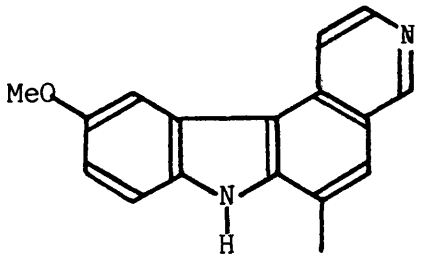
(155a)

Another foreseeable problem in the synthesis of olivacine and its derivatives (R_{11}=H) by this method is that there are two potential sites for the final ring closure, namely the 2-and 4-positions of the carbazole.



(155b)

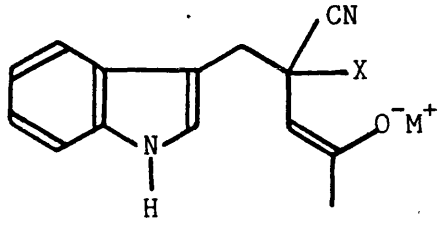
Reassuringly it has recently been reported[116] that olivacine has been successfully synthesised from 3-acetyl-1-methyl-carbazole using this final cyclisation in good yield with no mention made of the other potential product. In contrast Viel[117] has reported that the cyclisation of the related amine (where R_5=OMe) gave a 20% yield of the pyrido[3,4-c]-carbazole by-product (156).



(156)

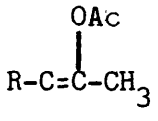
If this non-selectivity constitutes a major problem, it may be solved earlier in the synthetic route.

If compound (140) is taken when R_5=H, it should be possible to preferentially form the thermodynamically more stable secondary enolate (157) under equilibrium conditions and trap it as the enol acetate.

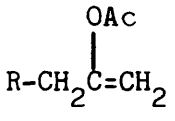


(157)

Work by House and Trost[118] and House and Kramar[119] have obtained results of > 90% selectivity of the thermodynamic enol acetate for methyl alkyl ketones.



THERMODYNAMIC

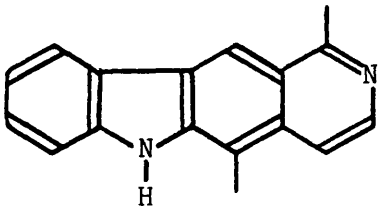


KINETIC

This enol acetate (157) could then be alkylated to give a handle for the cyclisation of the final pyridine ring and thus remove the problem of ambiguity in the final structure.

RESULTS

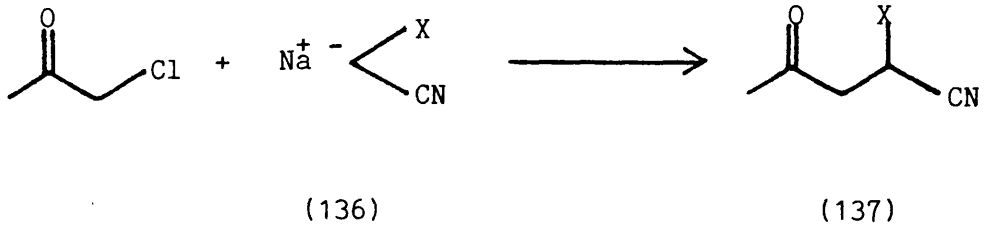
To examine the potential of the route set out in the previous section of this thesis, a synthesis of olivacine (95) was undertaken as the simplest case.



(95)

Olivacine

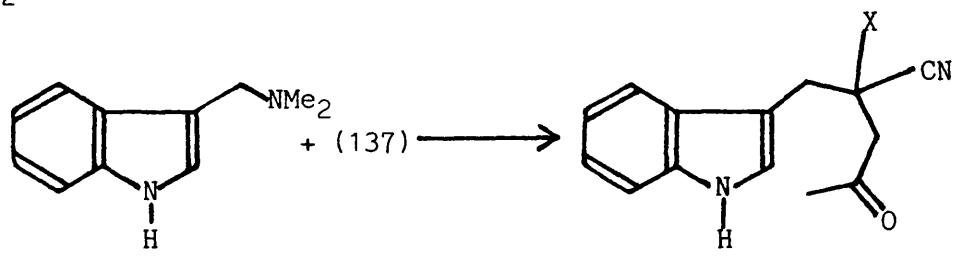
Our proposed route for the synthesis of olivacine is detailed as follows:-



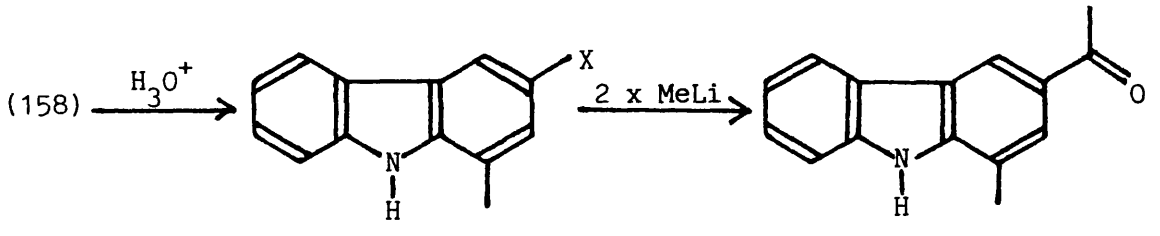
(136) (137)

(a) X=CO_2Et

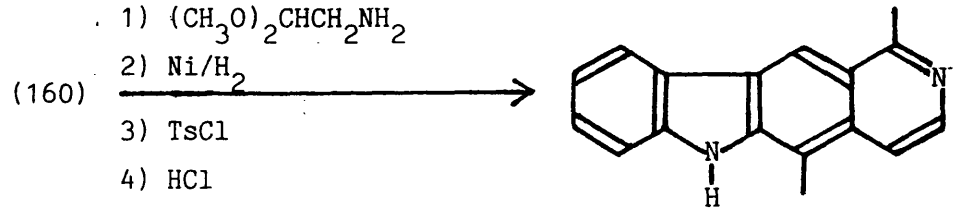
(b) X=CN



(139a) (158)



(159) (160)



(95)

SCHEME 61

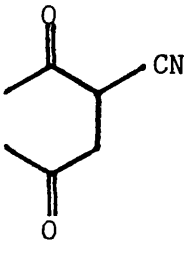
However, as the last step from (160) to (95) has already been successfully carried out[116], with a 65% yield from the N-tosyl derivative, a new synthesis of carbazole(160)was considered sufficient to prove the viability of the route.

For the first step of the scheme, we required a 2-substituted 4-oxopentanonitrile (137), where X is an electron withdrawing function, which can be converted at a later stage to an acetyl group. Two compounds, chosen as potentially useful, were ethyl 2-cyano-4-oxopentanoate (137a) (X=CO_2Et) and 2-cyano-4-oxopentanonitrile (137b) (X=CN).

Ethyl 2-cyano-4-oxopentanoate (137a) has been previously made[77] by a reaction between the sodium salt of ethyl cyanoacetate and chloroacetone, and in our hands this afforded the required ester (137a) in 81% yield. Sodium ethoxide was used as base and ethanol as solvent.

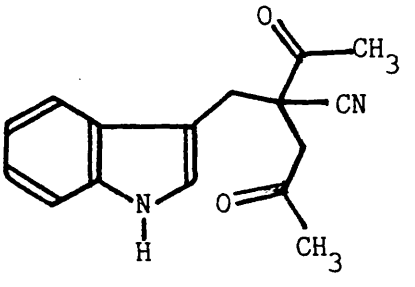
Although the synthesis of 2-cyano-4-oxopentanonitrile has not been reported before, there seemed no apparent reason why a similar condensation reaction between malononitrile (136a) (X=CN) and chloroacetone would not yield this substrate. However, when we first carried out this reaction under the same conditions as above, we found that the yield obtained was low, being only 26% after distillation. This was dramatically improved (to 87%) when sodium hydride was used to generate the sodio derivative of the precursor (136a) with dry THF as solvent.

It should be noted that for the quickest access to olivacine, 3-cyanohexane-2,5-dione (137c) (X=COMe) might be used, thus dispensing with the methylation of the carbazole (159) as discussed previously. This compound (137c) has been made by an adaptation of the above route[120] using chloroacetone and 3-oxobutyronitrile (136c) (X=COMe).



(137c)

In the general context, however, the utilisation of this starting material would not allow further alkylation processes, since, of course, there are now two acetyl groups present. Thus, if the indole (158c) were prepared, we would not be able to control its further elaboration, nor perhaps its selective ring closure.

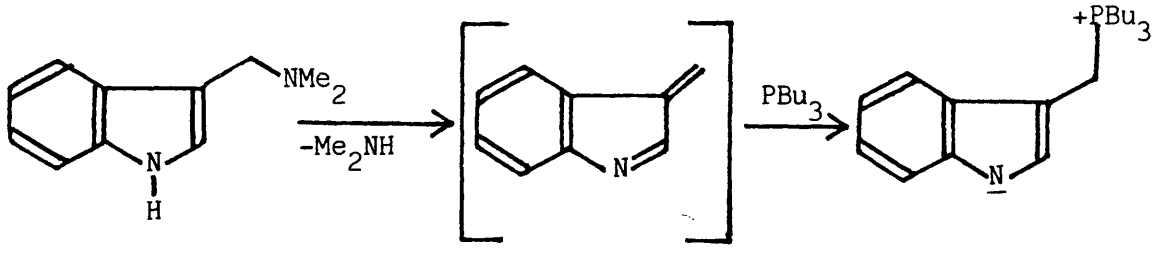


(158c)

As mentioned in the discussion section, gramine (139a) and its quaternary salts have been used for many years as a method of introducing an indole-3-methylene moiety into an active methylene compounds. Various catalysts, solvents and conditions have been used for this purpose. Some, but not all,of these methods were investigated with a view to optimising the above reaction. A summary of the results is recorded in Table 8.

An early concern was that the ketone (137) might undergo aldolisation in strongly basic conditions, or might expel hydrogen cyanide. For this

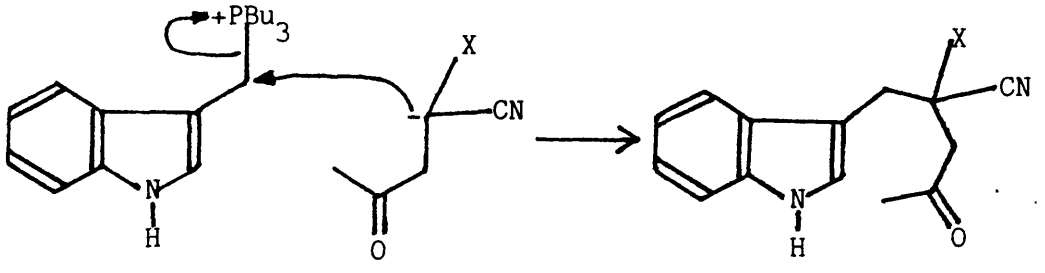
reason we initially chose one of the newer catalysts, namely tri-n-butylphosphine, because of its low basicity. The reaction probably follows the same mechanistic route as previously described, thus the methylene indolenine intermediate (147) generated by the elimination of dimethylamine, is trapped and stabilised by the phosphine as the betaine(161). (Scheme 62)



(139a) (147) (161)

SCHEME 62

The anion of (137) generated by protonation of the betaine (161), then attacks the protonated betaine with the elimination of tri-n-butylphosphine to yield (158). (Scheme 63)



(161) (137) (158)

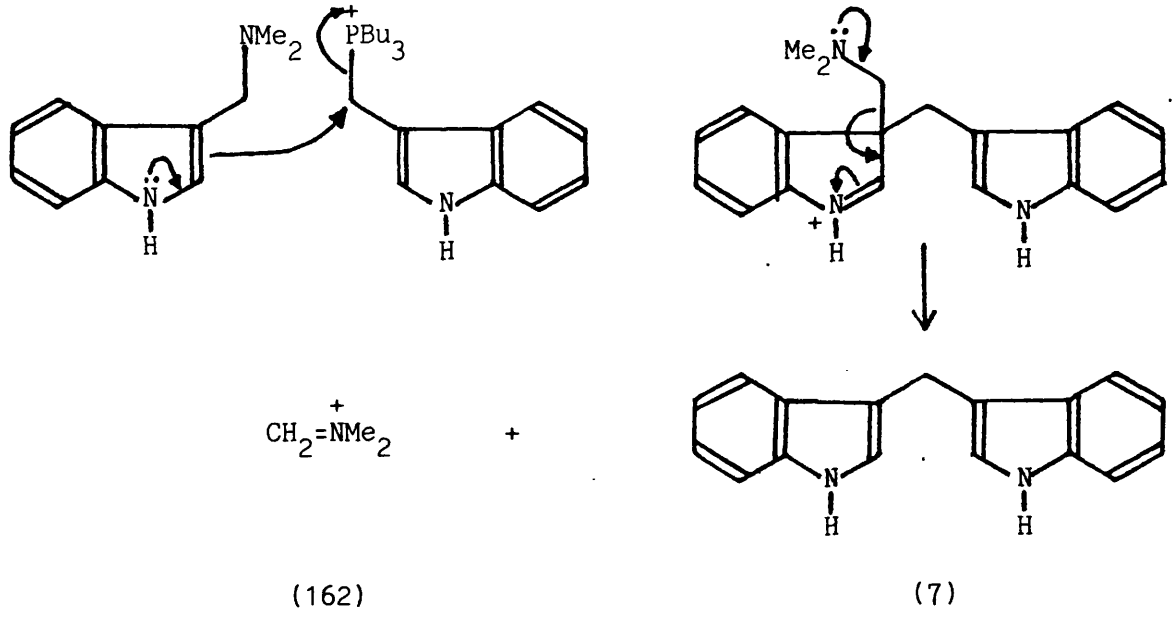
SCHEME 63

The reaction was first tried with (137a) on a small scale following the conditions detailed by Simei, Karasawa and Kaneko[91]. Thus gramine(139a) was heated under reflux with one molar equivalent of the ketone (137a) and 0.4 molar equivalents of tri-n-butylphosphine in dry acetonitrile under argon. The reaction was monitored by TLC, following the disappearance of the substrate(137a): the reaction was complete after 6 and 1/2 hours. Acidic work-up and chromatographic purification of the product gave the required indolyl-3-pentanone(158a)(X=CO_2Et) in 31% yield. From this result it was hoped that, if toluene was used as solvent, the higher available temperature would allow a more rapid conversion and fewer side reactions. Furthermore, we contemplated that, if a catalytic amount of p-toluenesulphonic acid were added after the alkylation step, cyclisation might also take place, and this could then be followed by aromatisation to the carbazole (159).

In practice a slight excess of gramine was used to promote the complete conversion of (137a) as it is cheap and easily removed by chromatography. The same relative quantity of (137a) and tri-n-butyl phosphine was used as before. A Dean-Stark trap was employed to remove any water produced by dehydration, thus preventing the reverse equilibrium step. After four hours at reflux, no water had been produced. TLC, however, showed an additional spot less polar than that of compound (158a). The reaction was continued, eventually overnight with regular TLC monitoring, but no apparent increase in the relative concentration of the new product was seen. At this time a trace of p-toluene sulphonic acid was added and the reaction continued for several hours, but with little or no effect on the results, as judged by TLC. The reaction was then repeated on a larger scale (3g of 137a) with again the same results.

Acidic work-up and chromatography gave pure (158a)(1 g) and a new product (0.85 g), which, to our dismay, turned out to be 3,3'-diindolylmethane (7), not the expected carbazole (159a).

Formation of 3,3'-diindolylmethane probably arises from attack through the β-indolic position of gramine onto either the protonated betaine or gramine itself and the subsequent elimination of the iminium species (162)or its equivalent. (Scheme 64)



(162) (7)

SCHEME 64

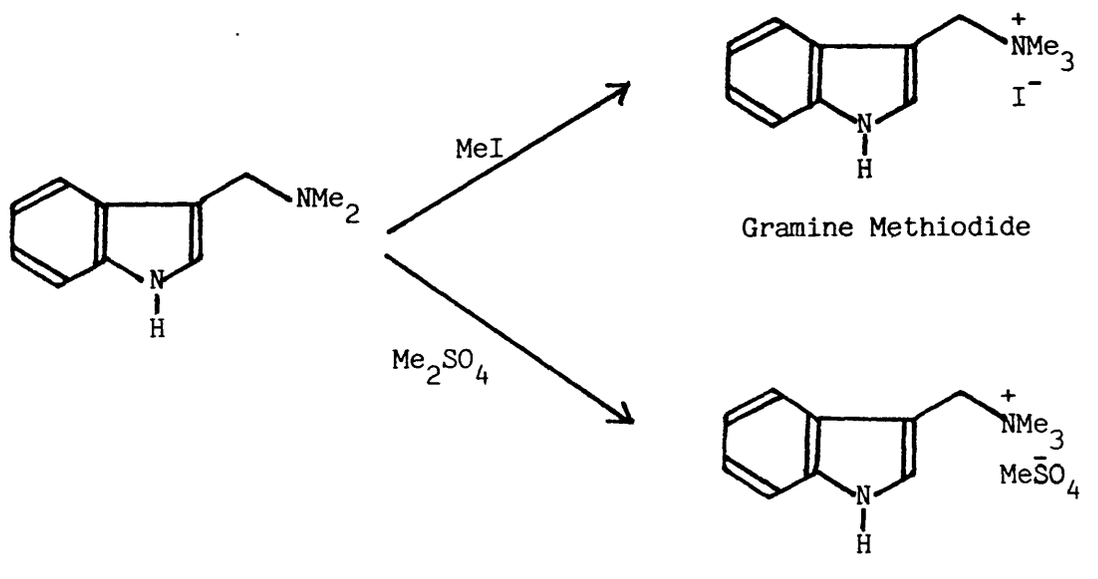
Certainly it is well established that indole-3-carbinol self-condenses by a mechanism of this type, yielding (7) and formaldehyde.

When the ketone (137b) was used, the indolylmethane (158b) was obtained free from 3,3'-diindolylmethane: the yield was 49%. This is still a poor result and so we looked for alternative procedures.

The next method investigated was that proposed by Howe, Zambite and Synder[82] using powdered sodium hydroxide as catalyst in dry toluene or xylene heated under a flow of nitrogen at reflux for several hours.

In two separate experiments the ketone (137b), gramine and a catalytic amount of powdered sodium hydroxide were heated at reflux in dry toluene for two and four hours. After work-up and purification these experiments gave the indolyl-3-pentanone (158b) in 56% and 62% yields respectively. The reaction was then repeated and its progress followed by the measurement of the evolution of dimethylamine in the exhaust gases. Unfortunately the emission lasted 120 hours, and after this time TLC of the resultant reaction mixture showed a complex mixture of products with no significant quantity of the desired compound (158b).

A reduction in reaction time to two hours, but now using xylene as solvent, was not particularly successful, and only 38% conversion to the indolylmethane (158b) was effected. A change of base to sodium hydride had no useful effect and so the application of gramine quaternary salts was considered, in particular, the methiodide and methiosulphate. These are formed quite simply by direct alkylation of the parent gramine. (Scheme 65).

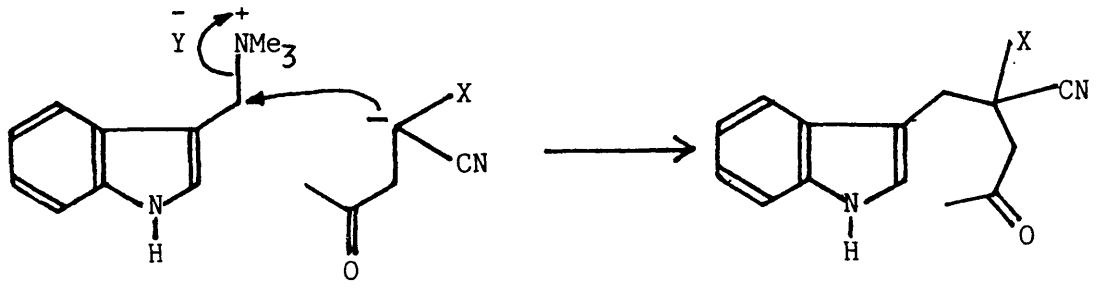


Gramine Methiodide

Gramine Methiosulphate

SCHEME 65

We anticipated that, should either of these two salts be reacted with the ketones (137) the elimination of trimethylamine would afford the indolylmethanes we required.



(137) (158)

SCHEME 66

The method originally adopted was that of Snyder, Smith and Stewart[121], where the quaternary salt was prepared in absolute ethanol and the sodium salt of the active methylene compound generated by the addition of sodium ethoxide. In our hands, this procedure gave disappointingly low yields for both the methiodide (10%) and methosulphate (11%), when the ketone (137b) was used as co-reactant.

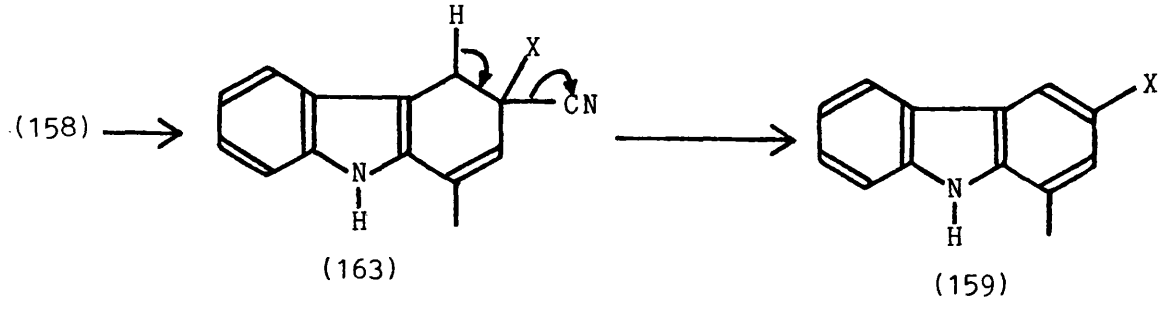
When the base was changed to sodium hydride and the solvent to THF however, yields were greatly enhanced. Gramine was dissolved in dry THF and the alkylating agent slowly added. The quaternary salt fell out of solution as a viscous gum. The anion of the ketones (137) were externally generated by treatment with sodium hydride in THF and then added to the reaction mixture, which was shaken until stirring was possible. Stirring was then continued overnight. By this method the indolylmethane (158a) was synthesised in 99% yield from the ketone (137a)

using dimethylsulphate as the quaternising agent. Because of the exceptionnally high yield of this reaction, no further work was deemed necessary using ketone (137a).

The yields for the dicyano derivative (158b) were, however, lower: 54% using the methosulphate and 40% for methiodide. Next HMPA was added to the gramine solution before the addition of the quaternising agent. It was hoped that this would not only improve the solubility of the quaternary salt itself, but also increase the activity of the sodio derivative of ketone (137b) by complexing the sodium counter ion. Yields of indolylmethane (158b) by this method were, however, only slightly higher at 41% for the methiodide and 66% for the methiosulphate.

Finally, a variation on the above method, previously used by Suter[92] and Holland[95], was tried. Here gramine and the sodium salt were combined, and the alkylating agent added slowly to the mixture. HMPA was again used as co-solvent. This gave the best result of all for the indolylmethane(158) (84%) using dimethyl sulphate as the quaternising agent. When methyliodide was used, however, the yield was only 36%. The results of all the reactions carried out using gramine and its quaternary salts are set out in tables 8 and 9 respectively.

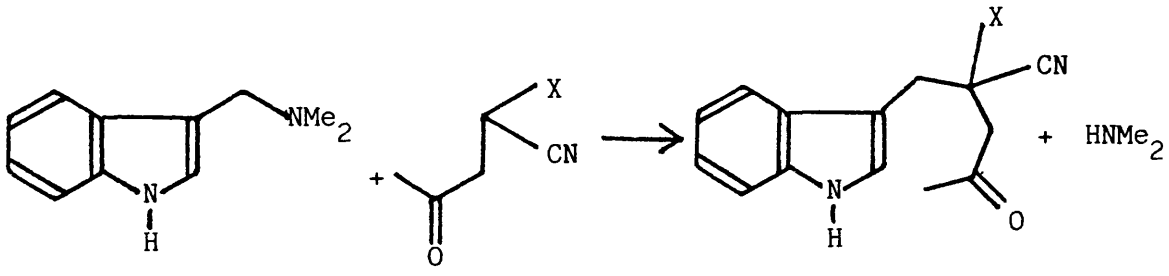
The next step in the synthesis of olivacine involves the acid catalysed cyclisation of indolylmethanes (158), followed by dehydration to the dihydrocarbazoles (163). It was then hoped that these products would eliminate hydrogen cyanide and aromatise to the carbazoles(159), perhaps spontaneously. (Scheme 68)



SCHEME 67

TABLE 8

Synthesis of the 4,4-disubstituted 5-(indol-3-yl)pentan-2-ones (158) from gramine (139a)



(139a) (137) (158)

SCHEME 68a

(158a) - ($X=CO_2Et$)

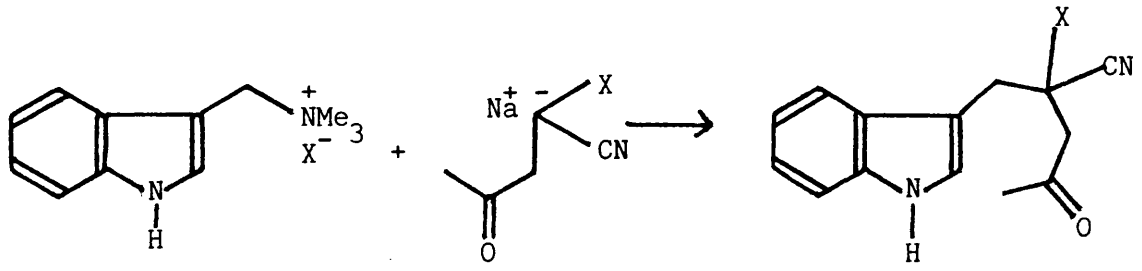
(158b) - (X=CN)

Catalyst	Compound Alkylated	Conditions Solvent/Temperature/Time	Product(s)	Yield %	Ref*
$P(nBu)_3$	137a	MeCN/Reflux/6+1/2 hrs	158a	31%	91
	137a	Toluene/Reflux/Overnight	158a(7)	19%(16%)	91
	137b	MeCN/Reflux/5+1/2 hrs	158b	49%	91
NaOH	137b	Toluene/Reflux/2 hrs	158b	56%	82
	137b	Toluene/Reflux/4 hrs	158b	62%	82
	137b	Toluene/Reflux/120 hrs	No Product		82
	137b	Xylene/Reflux/2 hrs	158b	38%	82

* Ref: a reference to the conditions used and not the product which in both cases has never previously been reported.

TABLE 9

Synthesis of the 4,4-disubstituted 5-(indol-3-yl)pentan-2-ones (158) from gramine quaternary salts



(137) (158)

(158a) - ($X=CO_2Et$)

(158b) - ($X=CN$)

SCHEME 68b

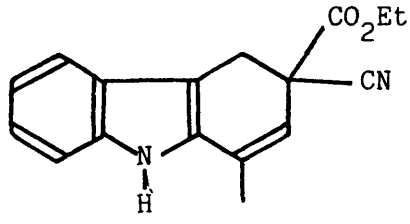
Method of Addition*	Anion Generator	Anion Species	Solvent	Quaternising Agent	Product	Yield %	Ref
a	NaH	137a	THF	Me_2SO_4	158a	99%	-
				MeI	Not attempted		
a	EtO^-Na^+	137b	abs EtOH	Me_2SO_4	158b	11%	121
				MeI	158b	10%	121
a	NaH	137b	THF	Me_2SO_4	158b	54%	-
				MeI	158b	40%	-
a	NaH	137b	HMPA/THF	Me_2SO_4	158b	66%	-
				MeI	158b	41%	-
b	NaH	137b	HMPA/THF	Me_2SO_4	158b	84%	-
				MeI	158b	36%	-

Note: all reactions stirred at room temperature overnight after addition.

*Method of addition:-

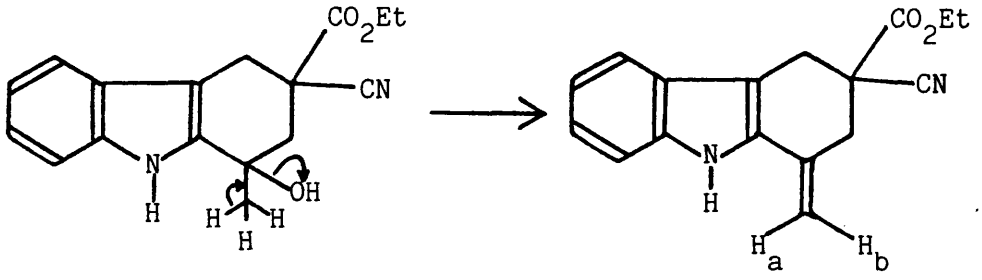
a) Quaternary salt generated and sodium salt added
b) Gramine and sodium salt mixed, then quaternising agent added.

The most commonly used catalyst for this type of cyclisation and therefore an obvious starting point is acetic acid. With this reagent at reflux, an attempt was made to cyclise our indolylmethane (158a) The spectral data for this product were originally most confusing. The infra-red spectrum showed the remaining presence of both nitrile and ester moieties. The mass spectrum showed a major peak at m/z 280, which corresponds to the loss of water from compound (158a). Both sets of data suggest that the product is (163a).



(163a)

The ^{1}H nmr spectrum, however, although confirming the presence of the ethoxycarbonyl group, showed retention of both the chain methylene groups of the precursor (158a), two, and not one, olefinic protons and no methyl group. The only explanation for these spectral data is that dehydration of the cyclised alcohol had occurred through loss of a terminal proton producing an exocyclic double bond, and thence the isomeric structure (164a). (Scheme 69)

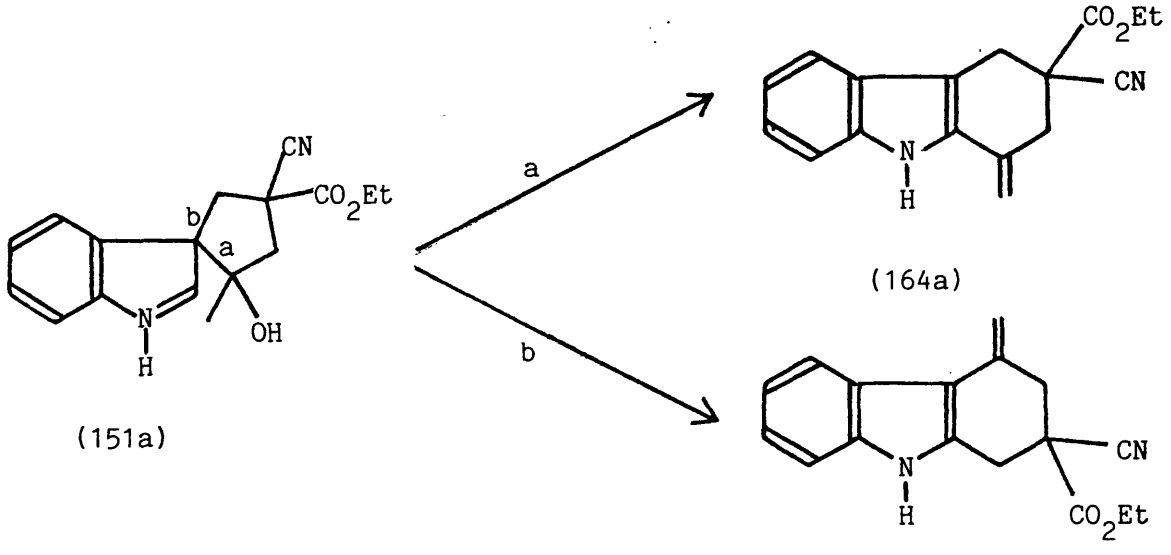


(164a)

SCHEME 69

A possible explanation for the occurence of this unexpected product may be the slightly smaller steric interaction between the indole nitrogen proton and H_a of structure(164a) compared to a similar interaction with the isomer(163a). A comparison of models, is, however, not very convincing.

The above interpretation of the data assumes that, if the reaction proceeds via a 3,3'-spirocyclic indolenine intermediate(151a), then the newly formed bond (a) and not (b) migrates. If the latter occurred, the isomeric product (165a) would form. (Scheme 70)



(151a)

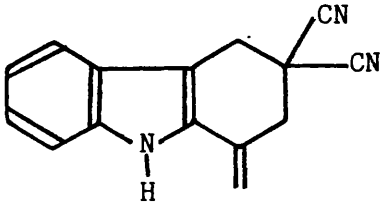
(164a)

(165a)

SCHEME 70

To check the authenticity of structure(164a), a nuclear Overhauser effect (n.o.e.) difference ^1H nmr was taken. The compound was irradiated at the indole nitrogen proton frequency. Should the compound have structure(164a), then the signal of one of the two olefinic protons (H_a) should, because of its proximity, be enhanced. With compound (165a), this would not be the case. The results of this study confirmed 3-cyano-3-ethoxycarbonyl-1-methylene-1,2,3,4-tetrahydrocarbazole (164a) to be the product, since an enhancement of 5 % was observed between the NH signal (8.32 ppm) and a resonance at 5.20 ppm - clearly an olefinic signal.

When the other indolylmethane (158b) was heated with glacial acetic acid under reflux, the same type of exocyclic structure(164b)was obtained in 21% yield.



3,3-dicyano-1-methylene-1,2,3,4-tetrahydrocarbazole

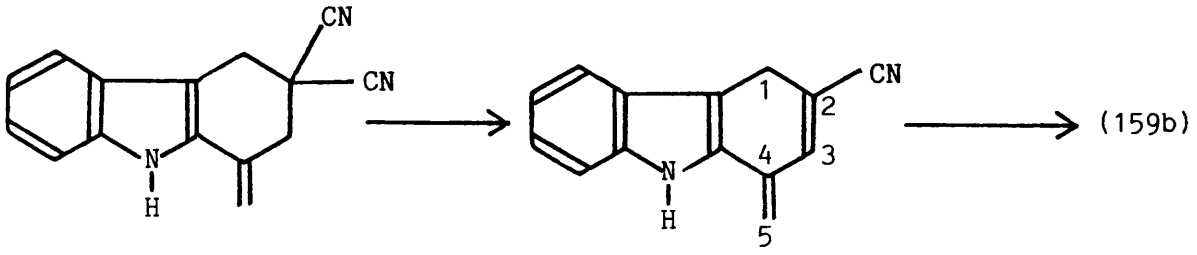
(164b)

Significantly, yields of both carbazoles (164a) and (164b) were greatly enhanced when 50% aqueous acetic acid was used as the cyclising agent; when ketone (158a) was heated to reflux in 50% aqueous acetic acid, the conversion to (164a) proceeded very cleanly. After six hours the reaction was stopped and yielded 91% of the pure carbazole(164a). The corresponding yield for (164b) varied between 70% - 76% using the same method.

Boron trifluoride-etherate, p-toluenesulphonic acid, polyphosphonate ester, as well as strong and weak acid resins were also tried as alternative catalysts for cyclisation, but only gave yields of between 0 - 44% for either substrate. All these experiments are summarised in Table 10.

Although the production of the exocyclic olefinic tetrahydrocarbazole (164) was unexpected, it presented no major problems to us, as in the case of (164b) at least we were able to convert it smoothly to the required carbazole in reasonable yields by simple thermolysis. However, the heating of our compound with bases in high boiling solvents, which was originally tried, had no effect.

A typical thermolysis was as follows: 3,3-dicyano-1-methylene-1,2,3,4-tetrahydrocarbazole(164b) was absorbed onto silica and heated to 250°C under a stream of nitrogen for one hour. On extraction and chromatography the carbazole (159b) was obtained in 54% - 87% yield. The reaction, whether ionic or radical, proceeds via the elimination of hydrogen cyanide and a subsequent 1,5-hydrogen shift to afford the more stable, fully aromatised carbazole (159b). (Scheme 71)

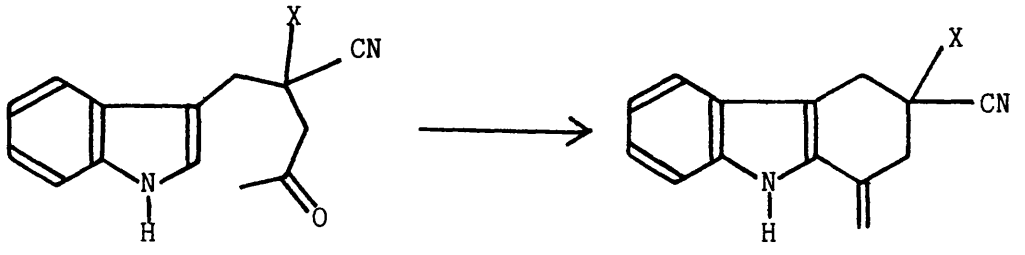


(164)

SCHEME 71

TABLE 10

Cyclisation of 4,4-disubstituted 5-(indol-3-yl)pentan-2-one(158)



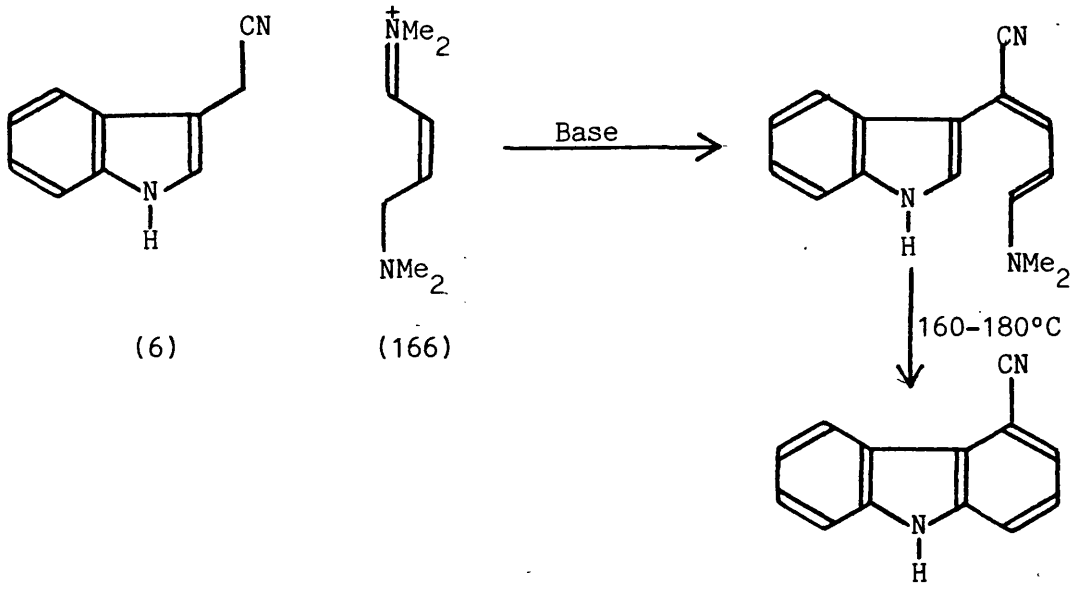
(158) (164)

(a) X=CO_2Et

(b) X=CN

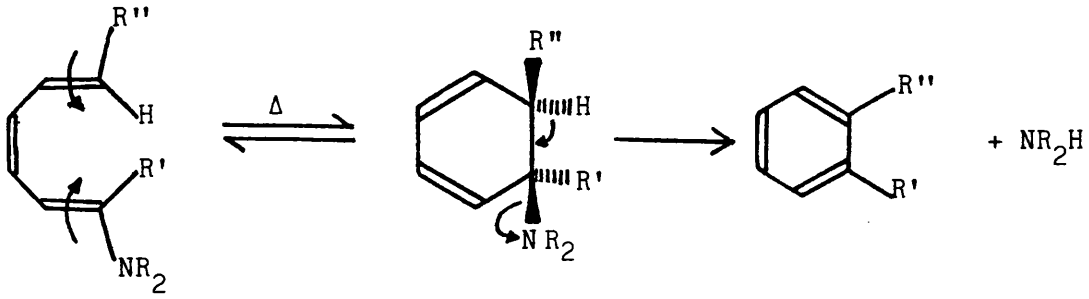
Catalyst	Indole	Conditions	Production	Yield %
AcOH	158a	Glacial AcOH/Reflux O/N	164a	42%
	158b	Glacial AcOH/Reflux O/N	164b	21%
	158a	50% aq AcOH/Reflux/6hrs	164a	91%
	158b	50% aq AcOH/Reflux/various	164b	70-76%
	158b	1 equiv AcOH,Toluene/Reflux/O/N	No reaction	
BF_3Et_2O	158b	Dichloromethane/RT/2+1/2hrs	164b	20%
pTSA	158a	Toluene/Reflux 1/2hr	164a	22%
	158a	Toluene/Reflux 4hrs	Complex mixture	
PPE	158b	5 equiv PPE/$CHCl_3$/Reflux 1/2hr	164b	44%
Resin	158a	Amberlite IRC-50T (Weak acid)/ Dichloromethane/RT 3hrs	No reaction	
	158a	Amberlyst 15 (Strong acid)/ Dichloromethane/RT 0-3hrs	Complex mixture	

Finally a completely unrelated and potentially very useful method of converting(158) to the carbazole (159) was investigated. Jutz and Wagner[122] have shown that indole-3-acetonitrile (6) can be converted into 4-cyanocarbazole(167) in 72% yield. (Scheme 72)



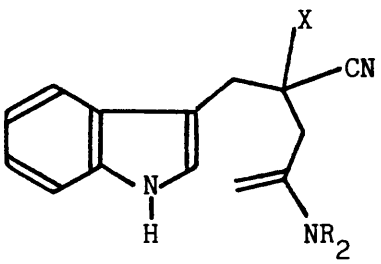
SCHEME 72

Thus if we were able to form the enamines of the ketones(158) and heat them, then the carbazoles(159)might form. This thermal cyclisation is a disrotatory process, and normally reversible. However, if there is a disubstituted amine on one of the terminal carbons of the hexatriene and a hydrogen atom on the other terminal, which exists in a trans-configuration relative to the amine in the cyclohexadiene, aromatisation can occur with elimination of the amine. This is an irreversible process. (Scheme 73)

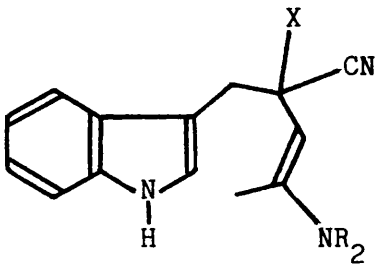


SCHEME 73

There are, of course, two potential enamines of the ketones (158) ie a primary species (168) and a secondary form (169).



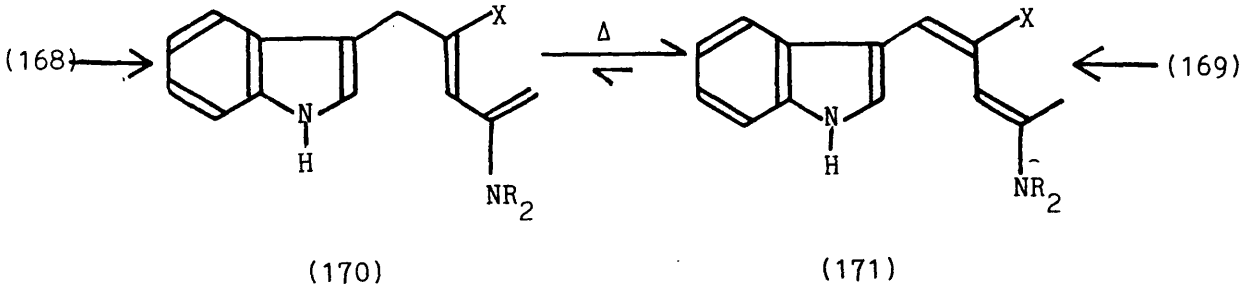
(168)



(169)

(X=CN,CO_2Et)

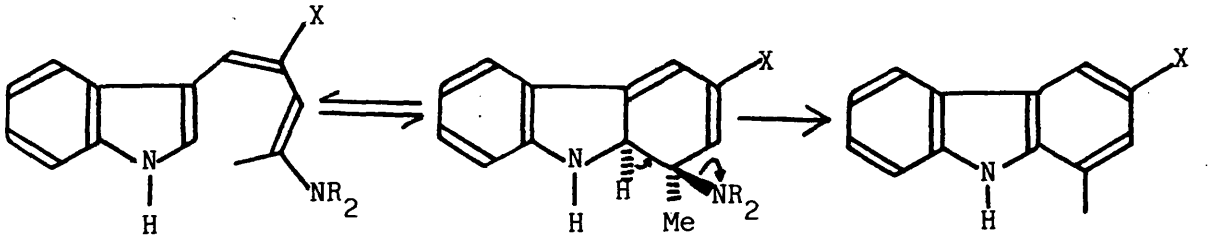
On heating, however, both (168) and (169) should eliminate hydrogen cyanide and yield the trienes (170) and (171) and thence the more stable isomer (171).



(170) (171)

SCHEME 74

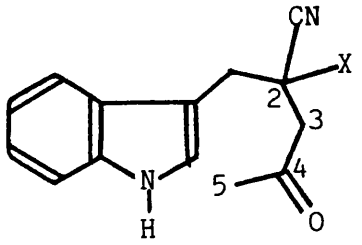
The triene (171) allowing for bond rotation to the cisoid isomers, was then set up to cyclise and eliminate the dialkylamine to yield carbazole (159). (Scheme 75)



(171) (159)

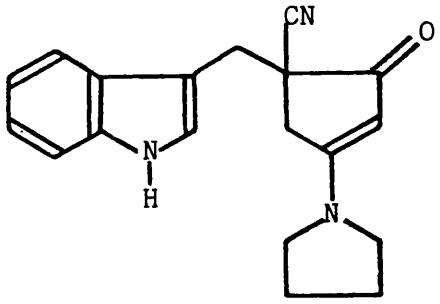
SCHEME 75

Also, if the enamines (168) and (169) could be separated, it would allow selective alkylations on the 3-or 5-positions of the chain which, for reasons previously stated, would be a useful feature.



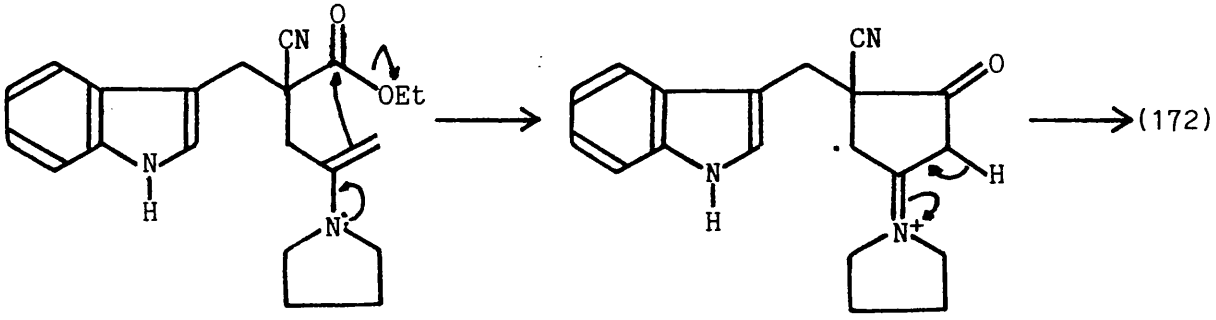
(158)

Reaction of (158a) with 2.5 equivalents of pyrol idine and a trace of p-toluenesulphonic acid in benzene at reflux for six hours yielded a single product. Spectral data for this product showed it not to be either of the enamines expected, but rather to be the cyclopentenone (172).



(172)

This compound(172)is brought about by the formation of the enamine (168a) ($X=CO_2Et$) and its subsequent attack on the ester moiety with elimination of ethanol. (Scheme 76)

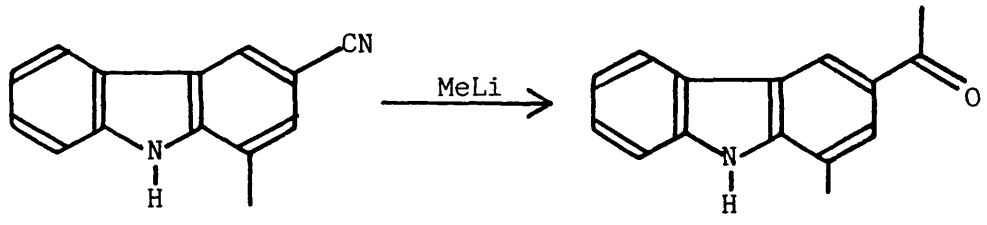


(168a)

SCHEME 76

Although this reaction is of no synthetic use, it does highlight a potential problem with the use of esters as X, when terminal anion reactions are being used. For this reason, further work with the ester moiety was dropped. Unfortunately, because of the time element, work on this approach had to be suspended.

The final step in our proposed synthesis toward olivacine (95) was the conversion of the nitrile (159b) to the ketone (160). This was successfully carried out using 2.2 equivalents of methyllithium to yield the required ketone in 83% yield. (Scheme 77)



(159b) (160)

SCHEME 77

EXPERIMENTAL

Ethyl 2-cyano-4-oxopentanoate (137a)

Sodium (5g, 0.22mol) was completely dissolved in absolute ethanol ($100cm^3$) with cooling when required. To this solution was then added ethyl cyanoacetate (136a) ($25cm^3$, 0.22mol) with the temperature maintained below 20°C. After addition and stirring at room temperature for a further 20 minutes, the resultant colourless suspension was slowly transfered, using a cannula, under nitrogen to a stirred solution of chloroacetone ($20cm^3$, 0.25mol) in absolute ethanol ($100cm^3$), again keeping the reaction temperature below 20°C. The resultant mixture was stirred for a further 30 minutes before the solvent was removed under reduced pressure and the residue partitioned between toluene ($100cm^3$) and water ($100cm^3$). The toluene extract was washed with water ($2 \times 100cm^3$), dried (Na_2SO_4), the solvent removed and the residue distilled by bulb to bulb distillation to yield a yellow oil (137a) (32g,87%), b.p. 80-85°C at 0.3mmHg (lit[77], 69-72°C at 0.01mmHg).

I.R. - ν_{max}(thin film)cm^{-1}; 2260(C≡N), 1745(CO_2Et), 1725(COMe)
lit. ν_{max}(CCl_4)cm^{-1}; 2260(C≡N), 1755(CO_2Et), 1733(COMe).

M.S. - (low eV, EI)m/z; 169(16%,[M^+]), 88(11%), 43(100%).

N.M.R. - δ_H($CDCl_3$)ppm; 4.17(2H,q(J=6Hz),$CO_2\underline{CH}_2CH_3$), 3.82(1H,t(J=6Hz),$\underline{CH}$), 3.05(2H,d(J=6Hz),$CH\underline{CH}_2$), 2.18(3H,s,$\underline{CH}_3CO$), 1.28(3H,t(J=6Hz), $CO_2CH_2\underline{CH}_3$).

2-Cyano-4-oxopentanonitrile (137b)

To a suspension of sodium hydride (1.2g, 50mmol) in dry THF ($30cm^3$) stirred under nitrogen and cooled in an ice bath was added, dropwise, a solution of malononitrile(136b)(3.3g, 50mmol) in dry THF ($10cm^3$). After addition, the ice bath was removed and the reaction mixture stirred for a further 30 minutes before the resultant suspension was slowly injected

via a cannula, into a cooled solution of chloroacetone (5.3cm^3, 66mmol) in dry THF (30cm^3). When the addition was completed, the ice bath was removed and the reaction mixture left to stir overnight. The solvent was then removed and the residue was purified by bulb to bulb distillation to yield a colourless solid (137b) (5.3g,87%), b.p. 220°C at 0.3mmHg, crystallised from a chloroform/petroleum ether (60-80°C), m.p. 55-57°C.

Elemental analysis - $C_6H_6N_2O$; requires C,59.00: H,4.95: N,22.94.
found C,58.57: H,4.97: N,22.52.

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 2200(C≡N), 1720(C=O).

M.S. - (Isobutane,CI)m/z; 123(M+1).
(70 eV, EI)m/z; 66(28%), 43(100%).

N.M.R. - $\delta_H(d^6DMSO)$ppm; 4.86(1H,t(J=6Hz),CH), 3.42(2H,d(J=6Hz),CH_2), 2.20(3H,s,CH_3).
- $\delta_C(d^6DMSO)$ppm; 202.8(s,C=O), 114.1(s,C≡N), 41.6(t,CH_2), 28.8(q,CH_3), 17.4(d,CH).

4-Cyano-4-ethoxycarbonyl-5-(3-indolyl)pentan-2-one (158a)

1) Tri-n-butylphosine as catalyst[91]

To a stirred solution of gramine (139a) (200mg, 1.15mmol) and ethyl 2-cyano-4-oxopentanoate (137a) (160µl, 1.15mmol) in dry acetonitrile (10cm^3) under a nitrogen atmosphere was slowly added a solution of tri-n-butyl-phosphine (100µl, 0.3mmol) also in dry acetonitrile (5cm^3). After addition the reaction mixture was heated to reflux with regular TLC monitoring. The reaction was seen to be complete after six and a half

hours, after which time the solvent was removed and the resultant gum acidified with 0.5N hydrochloric acid ($10cm^3$) and extracted with ethyl acetate ($2 \times 25cm^3$). The ethyl acetate layers were then combined, washed with water ($2 \times 25cm^3$) and brine ($25cm^3$), dried ($MgSO_4$) and the solvent removed. The resultant oil was then purified by column chromatography to yield a colourless solid (158a) (100mg, 31%) crystallised from dichloromethane/petroleum ether, m.p. 145-146°C.

Elemental analysis - $C_{17}H_{18}N_2O_3$; requires C,68.40: H,6.10: N,9.40.
found C,68.34: H,6.18: N,9.29.

U.V. - $\lambda_{max}^{nm}(\varepsilon)$; 289(4867), 281(5622), 221(14726).

I.R. - $\nu_{max}(Nujol)cm^{-1}$; 3480(N-H), 2255(C≡N), 1730(CO_2Et), 1710(COMe).

M.S. - (low eV, EI)m/z; 298(69%,[M^+]), 155(99%), 139(18%), 130(100%).

N.M.R. - $\delta_H((CD_3)_2CO)$ppm; 10.50-10.10(1H,br.s,exchanges on deuteration,NH), 7.50-6.70(5H,m,aromatic protons), 3.92(2H,q(J=6Hz),$CO_2CH_2CH_3$), 3.40-3.20(4H,m,2 x CH_2), 2.07(3H,s,CH_3CO).

The reaction was then repeated on the same scale using toluene as solvent, a Dean-Stark trap and 1.2 molar equivalents of gramine (139a). Although no water was produced after 4 hours, TLC monitoring showed the previous product (158a) had formed as well as a small quantity of a less polar compound which was hoped to be the carbazole (159a). Heating was continued for a total of 15 hours after which time the concentration of the less polar compound had considerably increased. A trace of p-toluene sulphonic acid was then added and heating continued in the hope that this would effect complete conversion to the expected carbazole (159a). When no changed in the reaction mixture was seen after 4 hours,

the solvent was removed and the reaction worked up as before. Chromatographic separation of the resultant mixture yielded the previous product (158a) (160mg, 47%) and a new product (50mg) which was shown to be 3,3'-diindolylmethane (7) (35%). The reaction was repeated on a larger scale (4g of gramine) with a reaction time of 15 hours, but no p-toluene sulphonic acid to yield compound(158a)(1g,20%) and 3,3'-diindolylmethane (7) (850mg, 40%).

2) From gramine quaternary salts[68]

To a stirred solution of gramine (139a) (8.75g, 50mmol) in dry THF ($50cm^3$) under a nitrogen atmosphere was slowly added a solution of freshly distilled dimethyl sulphate ($4.8cm^3$, 50mmol) in dry THF ($50cm^3$). On addition, the solution became cloudy and an insoluble gum rapidly formed of gramine methiosulphate.

In a separate flask a solution of ethyl 2-cyano-4-oxopentanoate (137a) in dry THF ($70cm^3$) was slowly added to a stirred suspension of sodium hydride (1.1g, 45mmol) also in dry THF ($30cm^3$) in an ice bath and under a nitrogen atmosphere. After the addition was complete, the ice bath was removed and the reaction mixture allowed to stir for a further 20 minutes. The resultant suspension was then transfered via a cannula into the methiosulphate reaction vessel, which was then shaken until stirring was possible. The mixture was then stirred for a further hour before the solvent was removed and the residue partitioned between chloroform ($50cm^3$) and 2N hydrochloric acid ($50cm^3$). The aqueous layer was washed with chloroform (2 x $25cm^3$), then discarded. The organic layers were combined, washed with water (2 x $50cm^3$) and brine ($50cm^3$), dried ($MgSO_4$), filtered and evaporated to yield an off-white solid (158a) (12g, 99%).

4,4-Dicyano-5-(3-indolyl)pentan-2-one (158b)

1) Tri-n-butylphosine as catalyst

The same conditions were used as for compound (158a). 2-Cyano-4-oxopentanonitrile (137b) (244mg, 2mmol) was reacted with gramine (139a) (348mg, 2mmol) in acetonitrile to yield, after 5 hours at reflux, an off-white solid (158b) (246mg, 49%). All attempts to crystallise this compound failed.

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 3460(N-H), 2200(C≡N), 1730(C=O).

M.S. - (low eV, EI)m/z; 251(75%,[M^+]), 130(100%), 122(34%).

N.M.R. - $\delta_H((CD_3)_2CO)$ppm; 10.40(1H,br.s,exchanged on deuteration,N-H), 7.70-6.90(5H,m,aromatic protons), 3.52+3.35(4H,s+m,2xCH_2), 2.10(3H,s,CH_3).

$\delta_C(CDCl_3)$ppm; 202.1(s,C=O), 136.1(s,indole C-8), 127.4(s, indole C-9), 125.6(d,indole C-2), 122.5(d), 120.3(d) 118.4(d), 116.0(s,CN), 111.9(d,indole C-7), 105.9(s,indole C-3), 47.5(t,CH_2), 34.4(s,$C(CN)_2$), 32.7(t,CH_2), 28.5(1,CH_3CO).

2) Sodium hydroxide as catalyst

Gramine (700mg, 4mmol), 2-cyano-4-oxopentanonitrile (137b) (430mg, 4mmol) and crushed potassium hydroxide (~50mg) were heated to reflux in dry toluene under a flow of nitrogen for two hours. The reaction mixture was then filtered while hot, the solvent removed and the residue purified by column chromatography to yield an off-white solid (158b) (500mg, 56%).

The reaction was then repeated with times of 4 hours (62%) and 120 hours (no product) using toluene as solvent and 2 hours using xylene (38%).

3) From gramine quaternary salts

Method A:

The gramine quaternary salts were prepared in dry THF ($10cm^3$) from gramine (700mg, 4mmol) and either methyl iodide or dimethyl sulphate (4mmol). To this was added the sodium salt of compound (137b) prepared from compound (137b)(430mg, 3.5mmol) and sodium hydride (85mg, 3.5mmol). The mixture was then shaken, if necessary, until stirring was possible, and left overnight. The solvent was then removed and the resultant gum partitioned between 2N hydrochloric acid ($25cm^3$) and ethyl acetate ($25cm^3$). The organic layer was then separated, washed with water ($2 \times 25cm^3$) and brine ($25cm^3$), dried ($MgSO_4$), evaporated and the resultant gum purified by column chromatography to yield the required compound (158b) in 54%, using dimethyl sulphate, and 40%, using methyl iodide.

When the reaction was repeated with HMPA ($1cm^3$) present in the gramine solution, before addition of the quaternising agent, the yields increase to 66% and 41% respectively.

Method B:

The sodium salt of 2-cyano-4-oxopentanonitrile (137b) (3.5mmol) was prepared as before in dry THF ($50cm^3$) and then a solution of gramine 700mg, 4mmol) in dry THF ($5cm^3$) and HMPA ($1cm^3$) added. The addition of

the quaternising agent (4mmol) then followed at a rate that prevented precipitation. After addition, the reaction was worked-up as above to yield compound (158b) in 84% (Me_2SO_4) and 36% (MeI).

3-Cyano-3-ethoxycarbonyl-1-methylene-1,2,3,4-tetrahydrocarbazole (164a)

1) Acetic acid

4-Cyano-4-ethoxycarbonyl-5-(3-indolyl)pentan-2-one (158a) (1g, 3.4mmol) was heated to reflux in glacial acetic acid ($50cm^3$) with regular TLC monitoring. As the reaction proceeded, the slow production of another less polar compound occurred. As after 5 hours there was a considerable proportion of the starting material (158a) still present, the reaction was kept at reflux overnight. TLC analysis of the resultant mixture showed all the starting material to have disappeared, so the solvent was removed and a solution of saturated sodium hydrogen carbonate ($50cm^3$) carefully added to the residue. This was then extracted with ethyl acetate (3 x $30cm^3$) and the aqueous layer discarded. The organic layers were then combined, washed with saturated sodium hydrogen carbonate (2 x $50cm^3$), water ($50cm^3$) and brine ($50cm^3$), dried ($MgSO_4$) and the solvent removed. The resultant residue was purified by column chromatography to yield an off-white solid (164a) (390mg, 42%), crystallised from diethyl ether, m.p. 133-135°C.

I.R. - $\nu_{max}(CHCl_3)cm^{-1}$; 3400(N-H), 2250(C≡N), 1742(C=O).

M.S. - (low eV, EI)m/z; 280(100%, $[M^+]$), 250(18%), 207(43%).

N.M.R. - $\delta_H(CDCl_3)$ppm; 8.32(1H,br.s, exchanged on deuteration,N-H), 7.50-7.10(4H,m,aromatic protons), 5.20(1H,s,olefinic proton

adjacent to indole nitrogen), 4.96(1H,s,other olefinic proton), 4.30(2H,q(J=7Hz)$CO_2\underline{CH_2}CH_3$), 3.56+3.38(4H,2s,2xCH_2), 1.31(3H,t (J=7Hz),$CO_2CH_2\underline{CH_3}$).

- $\delta_C(CDCl_3)$ppm; 168.1(s,$\underline{CO_2}Et$), 137.1(s), 131.4(s), 130.5(s), 126.8(s), 123.7(d), 119.9(d), 118.7(d), 114.9(s), 111.2(d), 109.1(s), 108.5(t,=CH), 63.1(t,$CO_2\underline{CH_2}CH_3$), 45.0(s,C-3), 38.6+30.1(2t,C-2+C-4), 13.9(1,$CO_2CH_2\underline{CH_3}$).

When the reaction was repeated using 50% aqueous acetic acid ($40cm^3$) with the same substrate (158a) and a reaction time of 6 hours, the yield of pure product (164a) was dramatically increased to 91%.

2) p-Toluenesulphonic acid

A solution of 4-cyano-4-ethoxycarbonyl-5-(3-indolyl)pentan-2-one (158a) (200mg) in dry toluene ($20cm^3$) was heated to reflux with p-toluene-sulphonic acid (~5mg). TLC analyses after 20 minutes suggested that nearly all the starting material had been converted to the required product (164a), so the reaction was stopped and the toluene solution washed with saturated sodium hydrogen carbonate (3 x $20cm^3$), water ($20cm^3$) and brine ($20cm^3$), then dried ($MgSO_4$). The solvent was then removed and the solid residue purified by column chromatography to yield an off-white solid (164a) (42mg, 22%) and a red gum (154mg) which was shown to contain a considerable amount of starting material. The reaction was then repeated on a slightly larger scale (500mg of (158b)) with an initial temperature of 20°C. No reaction was seen after one hour, so the temperature was raised to 40°C. Still, however, after a further hour at this temperature, no reacation had occurred, so the temperature was raised again to 80°C. After 20 minutes at this temperature, the con-version started to occur. On further heating, however, the proportion of

the starting material (158a) and product (164a) did not change, even after a further 4 hours, so the reaction was cooled and fitted with a Dean-Stark trap to remove any water which may have been causing the reverse reaction to occur. The reaction was then reheated to reflux for up to 15 hours, but still some of the starting material (158a) remained. As by this time a complex mixture of other products had formed, the reaction was not worked up.

3) Resins

The reactions were carried out by stirring the ketone (158a) (50mg) in dichloromethane ($5cm^3$) with the required resin at room temperature. Two resins were employed: the first Amberlite IRC-50J, a weak acid resin, had no effect on the substrate (158a) and the second Amberlyst 15, a strong acid resin, afforded a complex mixture of products of which none were the required tetrahydrocarbazole (164a).

3,3-Dicyano-1-methylene-1,2,3,4-tetrahydrocarbazole (164b)

1) Acetic acid

The reaction conditions were those used for 3-cyano-3-ethoxycarbonyl-1-methylene-1,2,3,4-tetrahydrocarbazole (164a) to yield, when glacial acetic acid was used, the required tetrahydrocarbazole (164b) in 21% yield, recrystallised from ethyl acetate/petroleum ether, m.p. 226-228°C.

Elemental analysis - $C_{15}H_{11}N_3$; Requires C,77.23: H,4.75: N,18.01

Found C,77.21: H,4.56: N,18.16

U.V. - λ_{max}nm(ε); 305(18,120), 212(22,400).

M.S. - (low eV, EI)cm^{-1}; 233([M^+]).

- (70eV, EI)cm^{-1}; 233(100%,[M^+]), 232(31%), 155(57%), 154(35%).

N.M.R. - δ_H($(CD_3)_2CO$)ppm; 10.40-10.60(1H,br.s,exchanged on deuteration, N-H), 7.60-6.92(4H,m,aromatic protons), 5.66(1H,s,olefinic proton), 5.20(1H,s,olefinic proton), 3.64+3.31(4H,2s, cyclic methylenes).

- δ_C($(CD_3)_2CO$)ppm; 138.4(s), 132.2(s), 130.3(s), 127.3(s), 124.4(d), 120.5(d), 119.5(d), 116.8(s), 112.1(d), 110.5(t,olefinic methylene), 168.1(s), 40.2+31.5(2t, cyclic methylenes)

The reaction was then repeated with 50% aqueous acetic acid as solvent over various reaction times to yield the required tetrahydrocarbozole (164b) after three and a half hours in 71%, four hours in 70%, five hours in 27%.

2) Polyphosphonate ester (PPE)

4,4-Dicyano-5-(3-indolyl)pentan-2-one (158b) (700mg, 2.8mmol) was heated at reflux with polyphosphonate ester (3 equivalent) in chloroform as solvent ($30cm^3$). After 30 minutes TLC suggested that the reaction had gone to completion, so the solvent was removed and the residue stirred with water ($50cm^3$) for 30 minutes before being extracted with ethyl acetate (2 x $30cm^3$). The organic fractions were combined, washed with water (2 x $30cm^3$) and brine ($30cm^3$), then dried ($MgSO_4$), filtered and evaporated. Column chromatography of the resultant gum yielded tetrahydrocarbazole (164b) (120mg, 19%) and an orange gum (600mg). TLC analysis of this gum showed it to contain a large proportion of the starting material (158b), so the reaction was repeated on this gum. Once again the required product started to form. After 5 hours the reaction was stopped and worked up as above to yield after chromatography compound (164b) (165mg), giving an overall yield of 44%.

3) Boron trifluoride etherate

To a stirred solution of 4,4-dicyano-5-(3-indolyl)pentan-2-one (158b) (200mg, 0.8mmol) in dichloromethane (10cm^3) under a nitrogen atmosphere was slowly injected neat boron trifluoride etherate (200µl, 1.6mmol). TLC monitoring showed the starting material to be slowly but clearly converted to the product (164b) over a period of two hours. The reaction was then stopped and carefully poured onto a stirred solution of saturated sodium hydrogen carbonate (10cm^3). After five minutes the chloroform layer was separated, washed with water (2 x 20cm^3) and brine (20cm^3), dried ($MgSO_4$), filtered and the solvent removed. Column chromatography of the resultant gum yielded the required product (164b) (38mg, 20%).

3-Cyano-1-methylcarbazole (159b)

3,3-Dicyano-1-methylene-1,2,3,4-tetrahydrocarbazole (164b) (350mg) was absolved onto silica (3.5g) and heated to 250°C under a stream of nitrogen. After 1 hour, ^{1}H nmr analysis showed the reaction to have gone to completion (loss of olefinic protons at 5.66 and 5.20ppm), so the reaction was cooled to room temperature, the silica was extracted with ethyl acetate (4 x 20cm^3). The organic layers were combined, evaporated and the resultant yellow solid purified by column chromatography to yield the required carbazole (159b) (195mg, 63%) as a colourless solid, crystallised from chloroform/petroleum ether, m.p. 193-194°C.

Elemental Analysis - $C_{14}H_{10}N_2$; Requires C,81.53: H,4.89: N,13.59

Found C,81.53: H,4.75: N,13.50.

U.V. - λ_{max}nm(ε); 274(45060), 242(28200), 232(29740), 216(26900).

I.R. - ν_{max}(Nujol)cm^{-1}; 3400-3300(N-H), 2200(C≡N).

M.S. - (low eV, EI)m/z; 206[M^+].

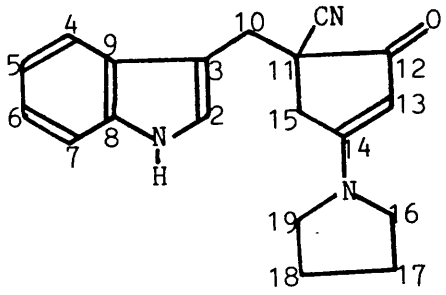
N.M.R. - δ_H($(CD_3)_2CO$)ppm; 11.08-10.63(1H,br.s,exchanges on deuteration,N-H), 8.04-7.82(2H,m,aromatic protons at 2- and 4-positions of ring), 7.42-6.82(4H,m,other aromatic protons), 2.50(3H,s,CH_3).

- δ_C($CDCl_3$)ppm; 139.4(s), 128.9(s), 127.0(s), 126.4(d), 125.6(d), 120.7(s), 120.4(d), 119.7(s), 119.5(d), 117.9(d), 111.2(s), 16.7(q,CH_3).

When the reaction was repeated using the same conditions, the yield of the carbazole(159b) ranged from 54-87%.

Attempted synthesis of the enamine of 4-cyano-4-ethoxycarbonyl-5-(3-indolyl)pentan-2-one (158a)

A solution of 4-cyano-4-ethoxycarbonyl-5-(3-indolyl)pentan-2-one (158a) (500mg, 1.7mmol), distilled pyrrolidine (0.35cm^3, 4.2mmol) and p-toluenesulphonic acid (50mg) in dry benzene (30cm^3) was heated to relfux for five and a half hours using a Dean-Stark trap. The reaction was then cooled, the solvent and pyrroline removed and the residue purified by column chromatography to yield an off-white solid (255mg) and some starting material (158a) (100mg). The new product was shown by spectral analysis to be cyclopentenone (172) (50%) and not the expected enamine m.p. 185-186°C (dec) crystallised from chloroform/petroleum ether.



(172)

I.R. - ν_{max}(Nujol)cm^{-1}; 3180(N-H), 2220(C≡N), 1658(C=O).

M.S. - (70eV, EI)m/z; 305(11%,[M^+]), 176(60%), 147(33%), 130(52%), 55(100%).

- (Iso-but,CI)m/z; 306(33%,M+1), 191(100%), 177(30%).

N.M.R.- δ_H(d^6DMSO)ppm; 11.00(1H,br.s,exchanges on deuteration,N-H), 7.60-6.70(5H,m,indole protons), 4.62(1H,s,H-13), 3.52-2.92 (8H,m,H-10,15,16,19), 2.10-1.72(4H,m,H-17,18).

- δ_C(d^6-DMSO)ppm; 192.7(s,C=O), 171.9(s,C-14), 136.5(s,C-8), 128.2(s,C-9), 125.0(d), 122.4(s), 121.5(d), 119.0(d). 118.9(d), 111.8(d), 108.6(s), 95.6(d,C-13), 49.7(t), 48.2(t). 47.9(s,C-11), 37.9(t), 31.7(t), 25.3(t),24.9(t).

3-Acetyl-1-methylcarbazole (160)

To a stirred solution of 3-cyano-1-methylcarbazole (159b) (100mg, 0.5mmol) in dry THF (5cm^3) at -80°C under a nitrogen atmosphere was added a solution of methyllithium (1.1mmol) also in dry THF (1cm^3). After addition

was completed, the reaction was allowed to warm slowly to room temperature over two hours with the precipitation of an orange solid. The reaction mixture was then cooled in an ice bath, methanol added ($1cm^3$) and the solvent removed. The resultant solid was stirred for ten minutes with 2N hydrochloric acid ($10cm^3$) and then extracted with ethyl acetate (2 x $10cm^3$). The organic layers were combined, washed with water (2 x $10cm^3$) and brine ($10cm^3$), dried, filtered and evaporated. This, however, only yielded 1-2mg of a yellow gum, so the aqueous and layer was neutralised with 2N sodium hydroxide to yield an orange precipitate. The precipitate was extracted with ethyl acetate (2 x $10cm^3$). The organic layers were then combined and treated as above to yield an orange solid (100mg). Spectral data suggested this to be the imine (93%) which had not hydrolysed as expected on treatment with 2N hydrochloric acid.

I.R. - ν_{max}(Nujol)cm^{-1}; 3650-3350(br.), 3255(sh.).

M.S. - (low eV)m/z; 223(52%,M+1), 222(100%,[M^+]),

N.M.R.- δ_H(d^6DMSO)ppm; 11.60-11.20(1H,br.s,indole N-H), 8.50(1H,s), 8.20(1H,m), 7.86(1H,s), 7.60-7.16(3H,m,rest of aromatic protons), 5.70-5.20(1H,br.s,C=N-H), 2.62+2.55(6H,2s, 2xCH_3).

The suspected imine (50mg) was then heated to reflux in 50% aqueous acetic acid ($5cm^3$) for 40 minutes. The solvent was removed and the residue treated with saturated sodium hydrogen carbonate ($10cm^3$), then extracted with ethyl acetate (2 x $5cm^3$). The organic layers were combined, washed with water (2 x $10cm^3$) and brine ($10cm^3$), dried ($MgSO_4$) and evaporated to yield a colourless solid (160) (45mg, 90%), crystallised from chloroform/petroleum ether, m.p. 193-194°C.

Elemental analysis - $C_{15}H_{13}NO$; Requires C,80.69: H,5.86: N,6.27

Found C,80.62: H,5.85: N,6.12.

U.V. - λ_{max}nm(ε); 328(5062), 288(12730), 272(14200), 236(13570).

I.R. - ν_{max}(Nujol)cm^{-1}; 3300(N-H), 1658(C=O).

M.S. - (low eV, EI)m/z; 223(70%,[M^+]), 208(100%), 121(26%).

- Accurate mass: $C_{15}H_{13}NO$; Requires - 223.0995

Found - 223.0975.

N.M.R. - δ_H(d^6DMSO/d^6Acetone)ppm; 11.19-10.87(1H,br.s,N-H), 8.58(1H,s), 8.10(1H,m), 7.80(1H,s), 7.60-7.10(3H,m,rest of aromatic protons), 2.64+2.62(6H, 2s, 2xCH_3).

- δ_C(d^6DMSO/d^6Acetone)ppm; 197.3(s,C=O), 141.7(s), 137.9(s), 129.9(s), 126.8(d), 123.0(s), 121.1(d), 120.3(d), 112.3(d), 104.1(s), 26.6(q,COCH_3), 17.2(q,ArCH_3).

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